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Observations on *Gastrodiscoides hominis* and *Fasciolopsis buski* in Assam.

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Published records of human infection with flukes in India are comparatively rare. These are concerned mainly with *F. buski* and *G. hominis*, and as these species are known to parasitise pigs as reservoir hosts, there has been a tendency to regard the sporadic human cases as infections accidentally incurred in areas where the flukes are endemic in pigs. To quote Chandler (1927) in connection with *F. buski* :—" With the single exception of the Manipur Valley in Assam, we feel confident that fluke infections do not exist in India except as sporadic cases. In the Manipur Valley *F. buski* has long been recognised as being of fairly frequent occurrence and we found it in 6% of 100 cases examined there. Sporadic cases of infection with this parasite also occur in Bengal, Bihar and Orissa, and in Madras Presidency. It is very common in Bengal and Assam pigs, which undoubtedly act as reservoirs for the infection."

With regard to *G. hominis*, Chandler found no cases of it in Assam. The majority of the few records of human infection with this species are, however, from Assam, namely, those of Lewis & McConnell (1876), Stephens (1906) and Giles (1890). Leiper's (1913) description of the new genus *Gastrodiscoides* was based on material sent by Dr. Mackie from Assam. Sharma (1933) recorded it in 3·2% of soldiers and civilians in Shillong. Although the number of cases of *G. hominis* infection in man is few, the number of worms found in certain individual cases has been large enough to justify the suspicion that this is not an accidental infection in man. Mackie (*vide* Leiper, 1913) stated he had seen as many as 200 worms passed after thymol treatment. Hodgson (*vide* Chandler, 1926) found over 700 worms in a single case. Bose (1934) obtained over 200 worms from two cases in Bihar and Orissa.

Fluke Infections in Assam.

Kamrup District occupies a section of the Brahmaputra river valley, or Assam Valley as it is called, and covers an area of over 3,000 sq. miles.

It is bounded on the north by the foothills of the Bhutan range and on the south by the Khasi and Jaintia hills. Its southern half is intersected by the Brahmaputra river running from east to west. Gauhati, the capital town and formerly the capital of Assam, is situated on the south bank of the river near the eastern boundary of the district. The whole district is flat and low-lying—Gauhati itself is only 170 feet above sea-level—and " bheels " or large shallow tracts of permanent standing water are a feature of its topography. During the rainy season this under-water area is increased enormously, making communications difficult except by boat and hundreds of villages become isolated. In the villages themselves in some cases the normal way of getting from house to house is by boat. This condition of things prevails from about May to October, but with the arrival of the " cold weather " the rainfall decreases rapidly, the water-level in the Brahmaputra and its tributaries throughout the district become markedly lower and dry conditions supervene.

Average monthly and annual rainfall (inches) over a period of 20 years in Kamrup District.

Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Average annual rainfall
·37	1·05	2·78	7·4	10·85	15·85	16·61	13·36	8·82	3·85	·47	·21	81·12

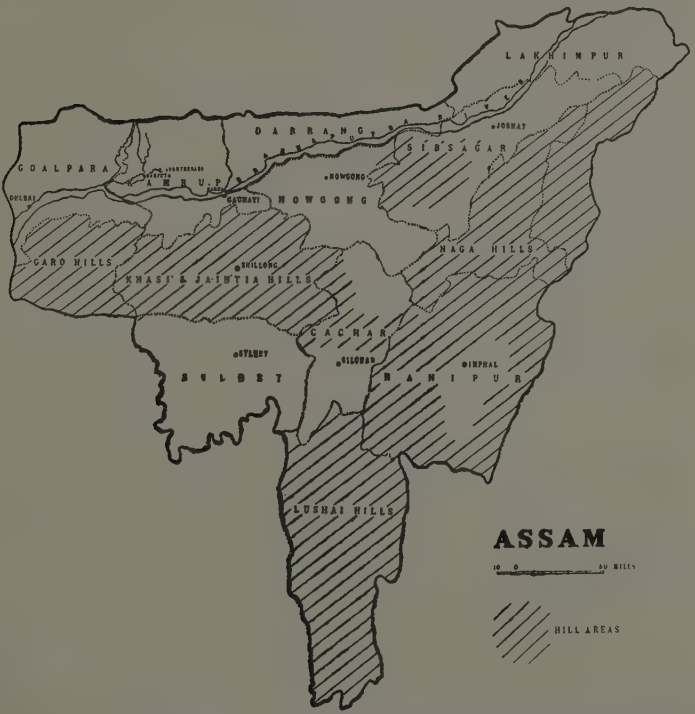
Average monthly and annual maximum and minimum temperature (°F.) over a period of 9 years in Gauhati.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Average annual temperature
Max.	74·1	77·7	85·5	86·7	87·8	89·2	90·4	89·9	89·9	87·3	81·8	75·3	84·6
Min.	49·7	51·9	59·0	67·3	71·9	76·2	78·0	77·8	76·7	70·5	60·5	51·3	65·9

The conditions in the district are therefore ideal for trematode dissemination. During the latter part of the rainy season or " hot weather " the snail population reaches its peak. The flooded villages and proximity of water to human habitations facilitate the contact and infection of snail vectors with human egg-bearing stools. Several kinds of edible water plants grow in abundance in the vicinity of the villages and undoubtedly

some of these act as transmitting agents for the infective trematode larvae to man.

Barka.—Whilst on a visit to Assam in 1934, the writer intended to go to Nowgong, whence Dr. Mackie's *G. hominis* cases came, in order to investigate there the extent of this and other human trematode infections. The occurrence of severe flooding in Nowgong district, however, made it



impossible. Information was then sought from the Pasteur Institute, Shillong, as to the sources of Col. Hodgson's *G. hominis* cases, and fortunately details of nine of these were available. These were concerned with Assamese from Kamrup district who had been admitted to hospital in Shillong in 1926 for kala-azar treatment, and as a result of routine treatment for hookworm, had passed numbers of *G. hominis* and in one case *F. buski* as well. Six of these cases were from Barka, a rather scattered village about 15 miles from Gauhati on the north side of the

river. Through the good offices of the Civil Surgeon, Kamrup, Capt. P. H. Cummins, I.M.S., the writer obtained faeces samples from 34 of the inhabitants of this village. Microscopical examination of these showed the following helminth infections :—

G. hominis	*F. buski*	*Ascaris*	Hookworm	*Trichuris*	Total examined
7	5	30	12	19	34

The remaining three cases in Col. Hodgson's list were from Katpuha, Baranghati and Sundardia respectively. The latter one of these proved to be one of the outlying villages of the large town Barpeta, which is situated somewhat centrally in the district.

Barpeta.—This town is an important trading centre for jute and rice and has a population of about 21,000. Being provided with a Dak Bungalow and a hospital it was a suitable place wherein to carry out investigation of helminth infections and work was begun here early in September, 1934. It was hoped to make a faecal survey of all the inhabitants of a small isolated village, consisting of about 20 houses, situated half-a-mile outside the town. With few exceptions, however, it was only possible to obtain faecal specimens from the smaller children. The result of examining eighty-one of these is as follows :—

G. hominis	*F. buski*	*Ascaris*	Hookworm	*Trichuris*	Total examined
50	62	66	3	39	81

A few of the more heavily fluke-infested children were brought to the hospital and treated with beta-naphthol. This drug was successful in eliminating *F. buski* in most cases but was ineffective against *G. hominis.* It was found that removal of *G. hominis* could be brought about in many cases by means of soap water enemas. One case, a boy of eight years, passed 790 *G. hominis* after the first enema and later in the day passed 199 more after another enema had been given, making a total of 989 worms. This method was not invariably successful, however. Presumably, in some cases the worms are situated too far up the lower bowel to be reached by the enema.

Sarthebari.—In October, 1934, a journey was made by boat from Barpeta to Sarthebari, a village of about 7,000 people, situated in very swampy " bheel " country 15 miles to the east of Barpeta. Here the writer spent sixteen days, and in exchange for gifts of small doses of beta-naphthol, obtained faecal specimens from 96 children. Here, as in

Barpeta and elsewhere in the district, the people are well acquainted with the appearance of "sothy," *i.e.*, *F. buski* and *G. hominis*, and "kechu" or *Ascaris*, and as there was no dispensary in the village, the offer of free worm-medicine was warmly received. The faeces examination gave the following results :—

G. hominis	*F. buski*	*Ascaris*	Hookworm	*Trichuris*	Total examined
29	58	79	7	18	96

The total number of people examined in Kamrup district for helminth infections was 221, and the percentages infected with the different kinds of helminths are as follows :—

G. hominis	*F. buski*	*Ascaris*	Hookworm	*Trichuris*	Total examined
41·2%	59·7%	81·9%	9·9%	34·8%	221

Experimental Work with *G. hominis* and *F. buski*.

In view of the frequency of human infections with these two flukes and the abundance of experimental material, work was undertaken in order to determine their life-cycles, in particular that of *G. hominis*, concerning which little is known. Most of the work therefore was directed towards discovering the molluscan intermediary of this species. Two lines of research were followed, (1) exposure of different species of water-snails to infection with miracidia of *G. hominis*, and (2) dissection of snails collected from infected villages in order to find natural infections with cercarial stages which might be identified with the adult.

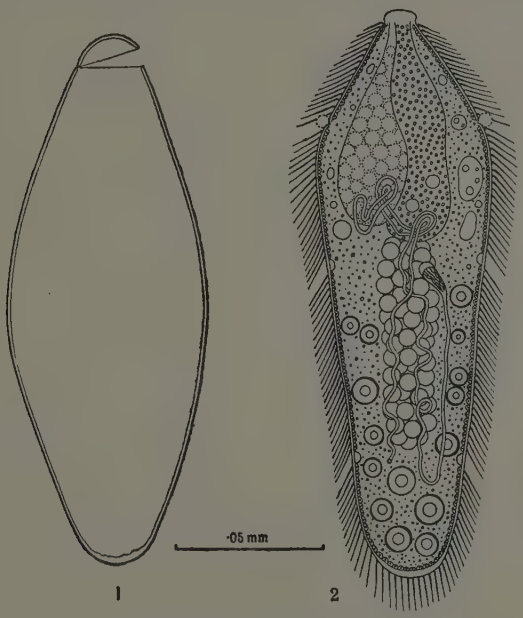
Method of making pure cultures of G. hominis *eggs*.—To obtain miracidia of *G. hominis* for experimental infections, cultures were made of eggs which had been dissected from the uteri of freshly passed adult worms. This source of supply, however, was so unreliable and the number of eggs so few that another method had to be found. Concentration of the eggs from faeces by sedimentation was then tried, and since it was very unusual to find a case in which only *G. hominis* eggs were present, selected cases were taken in which the numbers of *G. hominis* eggs appeared to exceed the *F. buski* eggs. It was noticed, however, that during the process of concentrating the eggs in such a stool, that is, during the repeated washing, sedimenting and decanting, the numbers of *G. hominis* eggs became relatively fewer and fewer instead of increasing, until after the final washing they were negligible in number compared with those of *F. buski*. The explanation of this disappearance of *G. hominis* eggs, discovered

accidentally, resulted in a method being elaborated for making pure cultures of these eggs. The loss of the eggs was the result of their adhesion to the surface of the water ; that is, although they have a higher specific gravity than water, they have the property, when they come in contact with the surface film, for example during the washing process, of remaining stuck to it. After sedimentation these eggs would be thrown away by decantation and so were lost. By means of a wire loop, however, they were saved and concentrated by removing the surface film after each washing. This " stickiness " of *G. hominis* eggs appears to be peculiar to them, and only very rarely was an *F. buski* egg seen with them at the surface.

G. hominis eggs measure 150–170μ long by 60–70μ in maximum breadth and are easily distinguishable from *F. buski* eggs by their shape, which is rhomboidal rather than oval, and by their colour, which is a pale greenish-brown unlike the yellow-brown of *F. buski* eggs. The operculum is more easily seen and the ab-opercular end of the shell is usually thickened. They develop more rapidly than *F. buski* eggs, the minimum time taken being 16–17 days at a temperature of 80 to 90°F.

The hatching of G. hominis *miracidium.*—The hatching of the miracidium was observed on several occasions. Prior to its emergence it lies with its anterior end adjacent to and pressed against the operculum. Large oil globules fill the space between it and the egg shell, but there is no mucoid plug between the shell and oral end of the miracidium as there is in *F. buski*, and it is directly in contact with the vitelline membrane at this point. The fully-developed miracidium shows characteristic movements inside the egg-shell, jerking its body spasmodically or contracting and elongating, while the cilia move in a leisurely manner. The flame cells are active and the excretory tubules are very distinct. About three minutes before hatching, the cilia in the anterior part of the miracidium become much more active, and just before emergence the sequence of events was as follows :—the miracidium contracted into the ab-opercular part of the egg leaving a considerable space between it and the operculum. Ciliar and body movements ceased absolutely and a rigid attitude was held for about 10 seconds, during which time it was noticed that the vitelline membrane was slowly coming away internally from the operculum, as though it were being pushed inwards by fluid permeating into the egg through the operculum joint. Minute granules were seen in this

space between the shell and the membrane. The latter was now becoming very faintly visible and appeared to be in the process of being dissolved. At this moment the operculum suddenly snapped open and a fraction of a second afterwards the miracidium leaped forward with its cilia now in violent action and surged out of the egg-shell. It was interesting to



Gastrodiscoides hominis.
1. Egg-shell. 2. Miracidium, lateral view.

note that just as in the case of *F. buski*, the opening of the operculum was not caused by the mechanical pressure of the miracidium from inside.

Morphology of the miracidium.—The miracidium of *G. hominis* is considerably longer than the egg and when swimming is typically "streamline" in shape, its maximum breadth being at the junction of the first and second quarters of the body. The cilia are long and in the anterior part of the body are arranged in orderly longitudinal rows, which are slightly staggered and give the body a rotary motion about its longitudinal axis when swimming. The terminal papilla is prominent.

The primitive gut is about one-third the length of the body and is displaced dorsally or ventrally by a secretory gland of about the same dimensions. An elongated mass of cells in the centre of the body represents the nervous system and/or glandular organs. The excretory system consists of two flame cells situated a little anterior to the middle of the body, from each of which a tubule runs posteriorly to the level of the excretory pores, then forward again to the anterior third where it loops three or four times and then back to the excretory pore which opens on a thickened band of non-ciliated cells which circles the body. The two excretory tubules follow parallel courses. There is another non-ciliated band of cells about the level of the flame cells. Around the anterior part of the miracidium is a constriction which appears to be the site of external glandular openings, but no ducts leading to it were seen.

In a culture containing free-swimming *F. buski* and *G. hominis* miracidia the two species could easily be distinguished. Not only by the absence of eye-spots were the *G. hominis* miracidia distinctive but also by their greater length and much more rapid and powerful swimming action. Although lacking eye-spots they are phototropic, like the miracidia of *F. buski*.

Exposure of snails to miracidia.—Cultures of *G. hominis* eggs having been made, every available species of water snail was collected from villages and elsewhere and were exposed to infection with the miracidia. The various species of snails collected and used are listed here.

Pila virens (Lam.)
Vivipara bengalensis Lam.
Planorbis exustus Deshayes
Limnaea acuminata Lam.
L. luteola Lam.
Bythinia pulchella Benson
Gyraulus compressus Hutton
Hippeutis umbilicalis (Benson)
Segmentina calathus (Benson)
S. trochoideus (Benson)
Melanoides tuberculata Muller
Thiara (*Melanoides*) *variabilis* Benson.
Hydrobioides turrita Neville
Paludomus conica Gray

With the exception of *Paludomus conica*, all these species were collected in the vicinity of infected villages, and all therefore had to be regarded as possible vectors. In the absence of any species of *Cleopatra*, which is believed to be the intermediate host of *Gastrodiscus aegyptiacus* and is, of course, an African genus, there was no biological clue as to which of these Indian species might be the vector of *G. hominis*. Accordingly, some indication in this direction was sought by means of preliminary

exposure of snails to miracidia in order to see if the latter would be attracted by or attack any one species in preference to others. Several different species of snails were put simultaneously in a Petri dish containing a large number of miracidia and were watched under a low power of the microscope, but they were not observed to be attracted by any one particular species. They would " swarm " around each snail, making tentative efforts at penetrating the soft parts but without success. They would adhere to the snail for a short time, with or without the rotating movement, and then swim away, or they would remain adherent for a longer period and finally drop off.

In the absence of any clue as to the probable vector all the species were exposed to the miracidia, kept alive for varying periods from 3 to 5 weeks during the hot weather and then dissected, but no developing stages or cercariae of the requisite type were ever found. Dissection of considerable numbers of snails collected at the three localities visited yielded natural infections with many different kinds of cercariae, but none of these could be suspected on morphological grounds to be the larvae of *G. hominis*.

Experimental Infection of *Segmentina* with *F. buski*.

In marked contrast to the negative experiments with *G. hominis* was the facility with which the minute snail *Segmentina trochoideus* could be infected with *F. buski*. This snail was first found in a large " tank " in Gauhati on the under-surface of water lily leaves, and was later found in Barpeta. Owing to its small size it was not easy to find or to obtain in large numbers. When placed in a vessel with *F. buski* miracidia, these were immediately attracted towards the snails and attacked them in large numbers, and in a short while a dozen or more would be seen adhering to the soft parts of the snails. By reason of the small size of these snails (2 to 3 mm. diameter) and the transparency of the shell and internal organs, developing stages of the infection could be seen in the living snails in strong transmitted light. Usually sporocysts could be detected 5 or 6 days after exposure to miracidia and of 30 snails exposed to infection, 15 were seen 10 days later to have become infected. The number of sporocysts resulting from exposure to miracidia were few, usually 3 or 4, compared with the large number of miracidia used for infection and it was surprising to note that hyper-infection with miracidia did not cause the death of the snails which apparently acquire some degree of immunity to the miracidia. Development through the redia stages

to cercariae was rapid. A snail infected for 27 days was seen to have two rediae in the liver, each of which contained numerous nearly mature cercariae. The minimum time observed between exposure to miracidia to emission of cercariae was 31 days. It was observed that the cercariae did not encyst either on the sides of the watch-glass or on blades of grass or fragments of water plants in the watch-glass, but died after a variable free-swimming period.

Like *Segmentina trochoideus*, the larger species *S. calathus* also showed a strong attraction for the miracidia of *F. buski*; but complete development in this species was not seen as the experimentally infected specimens died prematurely. It is, however, in all probability an intermediate host of *F. buski*.

Sources of Human Infection.

The final transmission of *F. buski* to man is known in the Far East to be effected through the agency of edible water plants and although encysted cercariae were not found on such plants in Kamrup, it may be taken for granted that one or more of the local species of water plants act as the infecting agents for both *F. buski* and *G. hominis*. The fruits of at least 5 different kinds of water plants are commonly eaten by the people in Kamrup and it was hoped by interrogation of children harbouring *F. buski* and/or *G. hominis* as to which kinds had ever been eaten by them, to obtain data which would point out the actual vector or vectors. It was found impossible, however, to form any conclusion from the information obtained as it was not reliable.

Specimens of the common species of water-plant whose fruits are eaten were collected and preserved for identification, and are listed here with their respective vernacular names.

Ottelia alismoides (L.) Pers. ... "Siantepa" or "Panical"
Nymphoea lotus L. ... ... "Moukwa"
Trapa natans Linn. ... ... "Singara"
Euryale ferox Salisb. ... ... "Nikhoru"
Nelumbo nucifera Gaertn. ... "Badam"

In addition to the fruits of these plants, a small tuber called "Helook" locally, probably the tuber of *Nymphoea* sp., is dug up from water-covered soil and is used for food.

From local information it is apparent that *G. hominis* and *F. buski* have a seasonal incidence. During the latter part of the cold weather and the beginning of the hot weather the demand for worm medicine at

the Barpeta dispensary is greatest. It appears that during this period the adult flukes must reach a peak of infestation for they are voided automatically and being easily perceived in the stool or as the result of vomiting (*F. buski*) give rise to the seasonal demand for anthelminthics. There is verbal evidence that this phenomenon reached epidemic proportions early in the year 1928 when an unusually large number of people was treated at the hospital and many of the outlying villages of Barpeta town suffered. Unfortunately no records were kept of the cases, but the prevailing species is said to have been *F. buski*.

The scarcity of pigs, due to the religious prejudices of the people against eating pig's flesh in Barpeta and the other villages where the flukes were common as human infections, is noteworthy. The writer was unable to obtain a single animal for post-mortem examination. On the other hand, pigs were easily obtainable in Cachar, in the tea-garden districts, where 30 were examined post-mortem for fluke infections but neither *F. buski* nor *G. hominis* was found. These flukes are also unknown as human infections in Cachar. From the scarcity of pigs in Kamrup district it may be concluded that they do not act as reservoirs for either *G. hominis* or *F. buski*, taking into consideration the widespread and common occurrence of these flukes in man in this locality. It is probable that the flukes are just as common in the adjoining districts, Goalpara, Darrang and Nowgong, which have a similar topography and native population. Possibly the whole of the Assam Valley is an autochthonous area. To the west the Brahmaputra river flows into Bengal where it joins the Ganges. There are no records of human infection with these flukes in Bengal, but they are commonly found in pigs in the abattoir at Calcutta. Bose's human cases of *G. hominis* farther to the west in Bihar and Orissa are therefore of much interest, assuming that these infections were incurred locally.

Summary.

1. *Fasciolopsis buski* and *Gastrodiscoides hominis* are recorded as common human infections in Kamrup District, Assam, where the examination of 221 faecal specimens from different localities gave an average infection rate of 59·7% with *F. buski* and 41·2% with *G. hominis*. In individual infections *G. hominis* may occur in very large numbers; 989 specimens were obtained on one occasion by means of soap water enemas which are effective in removing these worms.

2. Attempts to find the snail intermediary of *G. hominis* experimentally and by dissecting snails for natural infections were unsuccessful. The egg and miracidium are described. A list of water snails found in the endemic areas is given.

3. As a result of experimental infections, *Segmentina trochoideus* is recorded as a new intermediate host of *F. buski*. A list of edible water-plants which may possibly act as vehicles for the infective stages of the flukes in Kamrup is given.

ACKNOWLEDGMENTS.

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The Bone Marrow in Trichinosis of the Rat.

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Introduction.

Trichinosis is a disease which is characterised by profound absolute and relative variations in the leucocyte ratios of the circulating blood, more particularly a marked eosinophilia, and in moderate cases, concurrent neutrophilia (van Someren, 1938). It is obvious that these changes in the peripheral blood are the outcome of disturbances in the development of the precursor cells of the haemopoietic system, especially in the bone marrow; the lymphocytogenic tissue in the spleen and lymph glands does not appear to be so markedly activated, except in very severe cases or late in the course of the disease.

Beyond statements that the bone marrow is hyperplastic and rich in eosinophil myelocytes (MacCallum, 1932), no detailed account of the marrow changes in trichinosis is available. Opie (1904a) has stated that in natural eosinophilia of the guinea-pig the marrow shows myeloid hyperplasia, and there is a relative and absolute increase in the eosinophil precursor cells; these changes are also marked two weeks after infection with Trichinella, while in very severe trichinosis of the guinea-pig, where eosinopenia and aneosinophilia supervenes, Opie (1904b) has shown that eosinophiles and eosinophil myelocytes in the marrow are not increased, but show toxic degenerative changes and nuclear fragmentation. Spink (1934) has examined the bone marrow of trichinous guinea-pigs in which the blood eosinophilia had been reduced by complicating bacterial and protozoal infections, and found no reduction in the eosinophil elements under these circumstances, but no further details of the changes are given. The marrow changes in neutrophilia produced by infections with pyogenic organisms have been rather more fully studied, and since trichinosis in the rat produces both neutrophilia and eosinophilia (van Someren, 1938), the pathological changes in the marrow are particularly well marked.

Materials and Methods.

A series of eight adult white rats were infected with 500–600 encysted larvae, a dose which produces an eosinophilia of 13–20% in 7–16 days, and a neutrophilia of 70–80% 2–4 days after infection, which then falls and persists at about 45–50% for a considerable time (normal being 30–35%). Rats were killed at 13, 14, 21, 30 and 45 days after infection (during the acute stages of the disease) and the remaining three at 90 days after infection when encystment of the larvae had been fully completed. In addition, two normal uninfected rats, whose blood had regularly been examined for several months, were killed for marrow examination.

Marrow was obtained from the femur, which in the rat contains red marrow. Smears were made as for blood films, which were fixed in methyl alcohol, and stained for 2 hours or more in weak Giemsa (20 drops stain in 100 c.c. neutral distilled water). In addition the whole femur from one side of each rat was stripped of adherent muscle, fixed in Bouin, and then decalcified in 7·5% nitric acid in 5% formalin ; this was then sectioned longitudinally at 10μ, and the sections stained with haematoxylin and eosin.

Differential counts of several hundred cells were made on each smear, no distinction being made between metamyelocytes and myelocytes, and the architecture of the marrow was examined in the sections. Differential blood counts were also made on Giemsa-stained blood smears obtained immediately before killing the rat.

The Bone Marrow of the Normal Rat.

The femora of the normal white rat are completely filled with marrow of a dark red colour, soft and pulpy in texture. Microscopically the marrow is divisible into a dense medullary zone, which appears to be relatively avascular, and a peripheral zone, well marked at the proximal and distal ends of the femur, which contains numerous fat cells, between which are collapsed capillary channels and a reticulum of haemopoietic tissue (Plate I, Fig. 1). Erythroblastic and leucoblastic tissue are intimately mingled, there being no definite islands of either type, though it appears that the medullary zone is rather richer in erythroblastic cells, while cells of the myeloid series tend to be more numerous in the peripheral regions. Megakaryocytes occur indiscriminately in each region.

The table summarises the differential blood and marrow counts from the two control rats. Though lymphocytes are the most numerous leucocytes in the peripheral blood, lymphoid cells are practically absent in the marrow and no monocytes occur, unlike human marrow (Whitby and Britton, 1937). The smear is characterised by a myeloblastic aspect (Plate II, Fig. 5) though myeloblasts are not the most numerous type of cell. All types of granulocytes and their precursors are more numerous in the marrow than in the blood, and mature forms are slightly more numerous than the myelocytes. There appears however, to be no definite relation between the numbers of the myeloid cells in the marrow and those in the blood, nor could a definite relation be expected at any one time since the differential blood count of the rat varies widely from day to day (van Someren, 1938). The general relationship of the types of cells is, however, constant in both rats, and the proportion of cells of the myeloid series to nucleated red cells varies from 3 to 1 to 2 to 1.

Rat 2 was an animal showing a fluctuating low-grade eosinophilia common in many rats examined (van Someren, 1938), but it is interesting to note that the bone marrow appears quite normal, as are the other cell ratios of the blood. Characteristic of the rat (and mouse) are the number of neutrophiles and eosinophiles in the marrow showing ring-shaped nuclei; a few neutrophiles in the peripheral blood show this character, while it is common in the circulating eosinophiles; these are obviously young stages, and possibly correspond to a metamyelocyte stage in other mammals.

The Bone Marrow in Acute Trichinosis of The Rat.

Rats 3, 4, 5, 6, 7, were examined during the active stage of the disease. Macroscopically the femoral marrow shows little change except a tendency to be more pink in colour. Microscopically the marrow clearly shows functional myeloid hyperplasia; the medullary zone is not markedly altered, but in the peripheral zones and ends of the femur the hyperplasia is most evident, the fat cells having all disappeared, and dilated venous sinusoids being a prominent feature between the densely packed leucoblastic tissue (Plate I, Fig. 2). Mitotic figures are, however, not particularly numerous.

Smears from these rats have a myelocytic aspect (Plate II, Fig. 6), myeloblasts not being so evident. Total neutrophiles + neutrophil

myelocytes are slightly increased in most, and markedly increased in rat 6, where there was a persistently high blood neutrophilia, but mature marrow neutrophiles are actually reduced in these rats, indicating that the increase is taking place at the myelocytic level. There is again no exact relationship between the blood neutrophiles and the marrow neutrophiles, probably because of the fluctuating neutrophile percentage in the blood. The lower percentages than normal of mature marrow neutrophiles can be explained on the supposition that they are being liberated in the blood stream as soon as formed. That this is the case is shown by the fact that blood neutrophiles show a distinct left shift in the polynuclear count after infection (van Someren, 1938).

Basophiles and basophil myelocytes are reduced in all the infected animals, apparently taking no part in the general leucoblastic reaction.

The total eosinophiles + eosinophil myelocytes in the marrow is increased in all the infected animals showing eosinophilia, but the relationship is general only, and the total marrow count is usually less than the blood eosinophilia, with the exception of rat 5 ; in this case however, 11% was probably not the highest blood eosinophilia reached. Similarly, since the eosinophilia is a fluctuating quantity, and the maximum is reached at varying times after infection with the same infective doses in different animals, the blood counts given, which are those at the time of examination, may not indicate the true state of the reaction except for rats 6 and 8 where a complete record was kept. It is significant to note moreover, that formed eosinophiles (of the ring-nuclear type) are generally more numerous than myelocytes in the marrow, contrary to the findings of Opie (1904b), but it should be remembered that the infective doses given produce an eosinophilia considerably lower than the classical degree of eosinophilia in Trichinella infection (*e.g.*, up to 50% or more). With these higher degrees of eosinophilia the bone marrow picture may be different. In rat 6 the eosinophil reaction was not marked, as is evident in the blood and marrow counts, although autopsy revealed a heavy infection with living larvae in the muscles ; it is interesting to note, however, the marked neutrophil reaction in this case.

Premyelocytes are increased in all the infected animals, especially those showing a high blood eosinophilia, while myeloblasts are reduced ; this reduction is probably apparent only, being produced by the active increase at the premyelocytic-myelocytic level of leucopoiesis, and they are not

				Controls		Infected with 500–600 encysted larvae.							
Rat No.				1	2	3	4	5	6	7	8	9	10
Days after infection				—†	—*	13	14	21	30	45	90	90	90
Blood Counts %	Neutrophiles			31·1	35·7	43·0	31·5	42·0	52·5 (max. 73·0)	40·5	39·0 (max. 78·0)	54·5	49·0
	Lymphocytes			63·2	56·2	52·0	50·0	45·0	45·5	41·0	51·0	42·0	46·5
	Monocytes			3·4	3·0	—	2·0	2·0	—	2·0	2·0	2·0	4·0
	Basophiles			0·3	0·1	—	—	—	—	—	—	—	—
	Eosinophiles			2·5	5·0	5·0	14·5	11·0	2·0 (max. 6·0)	13·5	8·0 (max. 13·0)	1·5	0·5
Average blood count † over 120 days ; * over 75 days.													
Bone Marrow Counts %	Leucopoiesis	Mature granulocytes	Neutrophil	33·0	27·0	26·0	19·0	24·0	26·0	4·0	22·0	46·0	51·0
			Basophil	1·0	1·0	—	—	—	—	—	2·0	—	—
			Eosinophil	4·0	4·0	3·0	6·0	11·0	1·0	3·0	7·0	2·0	5·0
		Myelocytes	Neutrophil	28·5	26·0	40·0	53·0	39·0	58·0	57·0	43·0	33·0	16·0
			Basophil	1·5	—	1·0	—	—	—	1·0	—	—	—
			Eosinophil	1·5	2·0	7·0	3·0	5·0	2·0	8·0	3·0	7·0	2·0
		Premyelocytes		1·0	1·0	2·0	4·0	3·0	2·0	6·0	2·0	2·0	5·0
		Myeloblasts		4·0	4·0	5·0	—	2·0	—	3·0	4·0	6·0	7·0
		Lymphocytes		3·0	—	—	—	—	—	—	5·0	—	—
	Erythro-poiesis	Normoblasts		16·5	31·0	12·0	13·0	14·0	7·0	16·0	12·0	2·0	10·0
		Erythroblasts		4·5	2·0	2·0	2·0	2·0	3·0	2·0	—	2·0	3·0
		Megaloblasts		1·5	2·0	3·0	—	—	1·0	—	—	—	1·0

entirely absent. In all cases, however, it is evident that, as in the differential blood count, the differential marrow count varies from individual to individual with the same infective doses, and that atypical cases may be encountered in rats as in man (*e.g.*, rat 6).

Lymphocytes are absent in these infected animals, but as has been stated, they are not a constant element in the normal marrow.

The erythroblastic elements are very variable, even in the normal marrow, but it is evident that they are reduced in the infected animals, the ratio of myeloid cells to nucleated red cells varying from 8 to 1 to 5 to 1. Since a functional hyperplasia usually involves both leucoblastic and erythroblastic tissue (Whitby and Britton, 1937), this reduction is almost certainly relative only, especially since the total erythrocyte count of trichinosed animals shows no change (pig, Maass, 1933) or there may even be polycythemia with macrocytosis (rabbit, Wantland, 1937). Megakaryocytes show little change in infected animals.

The Bone Marrow After Recovery from Trichinosis in the Rat.

Depending on the individual animal, two distinct marrow pictures are encountered after encystment has been completed (3 months in these cases). In rat 8, the blood count had returned to normal, except for a persistent low-grade eosinophilia, which is generally the normal state after an attack. It is evident, however, that the marrow still shows the effect of stimulation, although the architecture is again normal (Plate I, Fig. 3). Neutrophil myelocytes are still more numerous than neutrophiles, although the total count neutrophiles + neutrophil myelocytes is almost normal; van Someren (1938) has shown that a left shift in the polynuclear count may persist for at least 2½ months after infection. At the same time eosinophiles and their precursors are also above normal, indicating continued stimulation, as may be recognised in the blood eosinophilia. Premyelocytes and myeloblasts are normal and the smear presents a normal aspect (Plate II, Fig. 7), although myeloid cells to nucleated reds are in the proportion of 7 to 1.

On the other hand rats 9 and 10 still show an abnormally high neutrophil blood count, though the eosinophiles are within normal limits. In these cases the marrow still appears hyperplastic (Plate I, Fig 4), but unlike the marrow in the high neutrophilia of the acute stages, the ratio of mature

neutrophiles to myelocytes is inverted ; the total neutrophile + neutrophil myelocytes count is also slightly above normal, but mature neutrophiles are more abundant than myelocytes, and the smear is characterised by an astonishing number of the ring-nuclear forms (Plate II, Fig. 8). It appears as if the production of neutrophiles has outrun the requirements, and great numbers of developed forms are being held back in the marrow, the excess above these being liberated into the blood stream to give the persistent abnormal count. At the same time eosinophil stimulation is still evident, eosinophiles and their precursors in the marrow being above normal, although the blood count is normal in this respect.

Premyelocytes and myeloblasts are slightly above normal, perhaps as a result of the excessive neutrophil reaction. Myeloid cells to nucleated reds are in the very high proportions of 24 to 1 in rat 9, and 6 to 1 in rat 10.

These changes bear little relationship to the severity of the muscle invasion, rat 8 being most heavily infected with 405 larvae per gram of muscle, while rats 9 and 10 showed 6 and 32 larvae per gram of muscle respectively ; a somewhat anomalous result, as the apparently more severe and prolonged reaction would be expected with the heavier infection. This appears only to emphasise the fact that the reactions depend on the individual host, and not necessarily on the severity of the infection.

This work was carried out while holding a Medical Research Council grant to Professor R. T. Leiper, F.R.S., to whom my sincere thanks are due. In addition I am indebted to Mr. W. R. Bale of the Department of Bacteriology, London School of Hygiene and Tropical Medicine, for valuable technical assistance in taking the photomicrographs illustrating this paper.

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PLATE I.

Fig. 1.—Haematoxylin-eosin stained section of normal femoral marrow (Rat 1); peripheral region, showing numerous clear spaces due to dissolved fat cells. (× 300.)

Fig. 2.—Haematoxylin-eosin stained section of femoral marrow in acute trichinosis (Rat 4); peripheral region, showing marked hyperplasia and dilated venous sinusoid filled with erythrocytes and polymorphonuclears. (× 300.)

Fig. 3.—Haematoxylin-eosin stained section of femoral marrow after complete encystment of larvae (Rat 8); peripheral region, showing return to normal disposition of fat cells. (× 300.)

Fig. 4.—Haematoxylin-eosin stained section of femoral marrow after complete encystment of larvae (Rat 10); peripheral region showing hyperplasia still evident. (× 300.)

PLATE II.

Fig. 5.—Giemsa-stained smear of normal femoral marrow of Rat 1. Myeloblastic aspect, showing portion of a megakaryocyte, a ruptured basophile, several mature neutrophiles and four myeloblasts (centre of field). (× 2000.)

Fig. 6.—Giemsa-stained smear of femoral marrow in acute trichinosis (Rat 4). Myelocytic aspect, showing small megakaryocyte, two mature eosinophiles, three eosinophile myelocytes, four mature neutrophiles and several neutrophile myelocytes and normoblasts. (× 2000.)

Fig. 7.—Giemsa-stained smear of femoral marrow of Rat 8, showing return to normal aspect; with two myeloblasts, an eosinophile myelocyte and several neutrophil myelocytes, mature neutrophiles and normoblasts. Scattered granules from a ruptured basophile. (× 2000.)

Fig. 8.—Giemsa-stained smear of femoral marrow of Rat 10, showing a myeloblast, a premyelocyte, several erythrocytes and numerous ring-nuclear neutrophiles. (× 2000.)

PLATE I.

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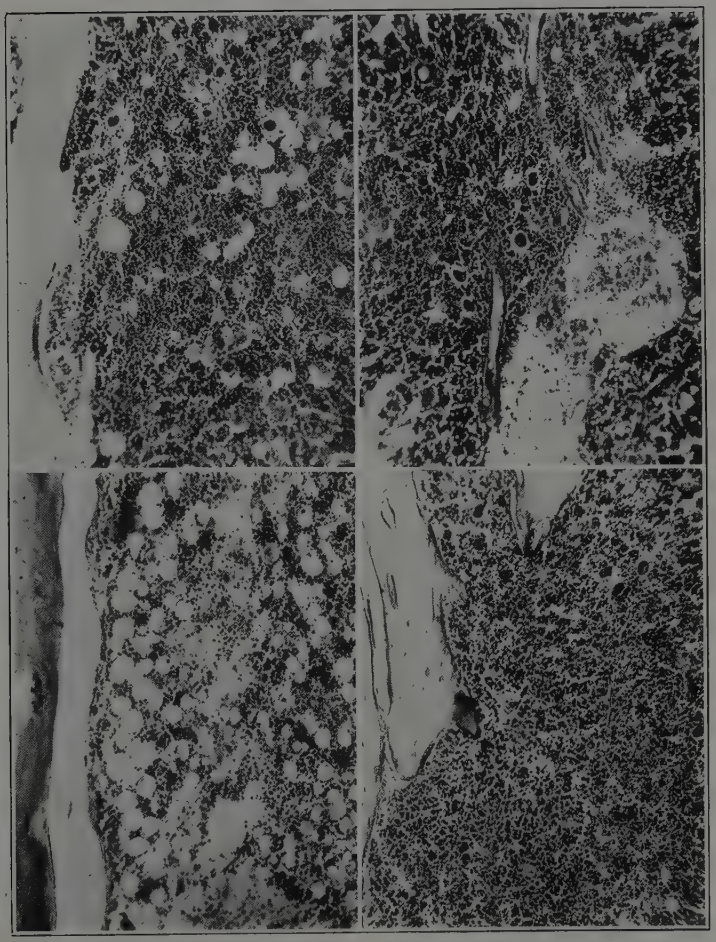

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PLATE II.

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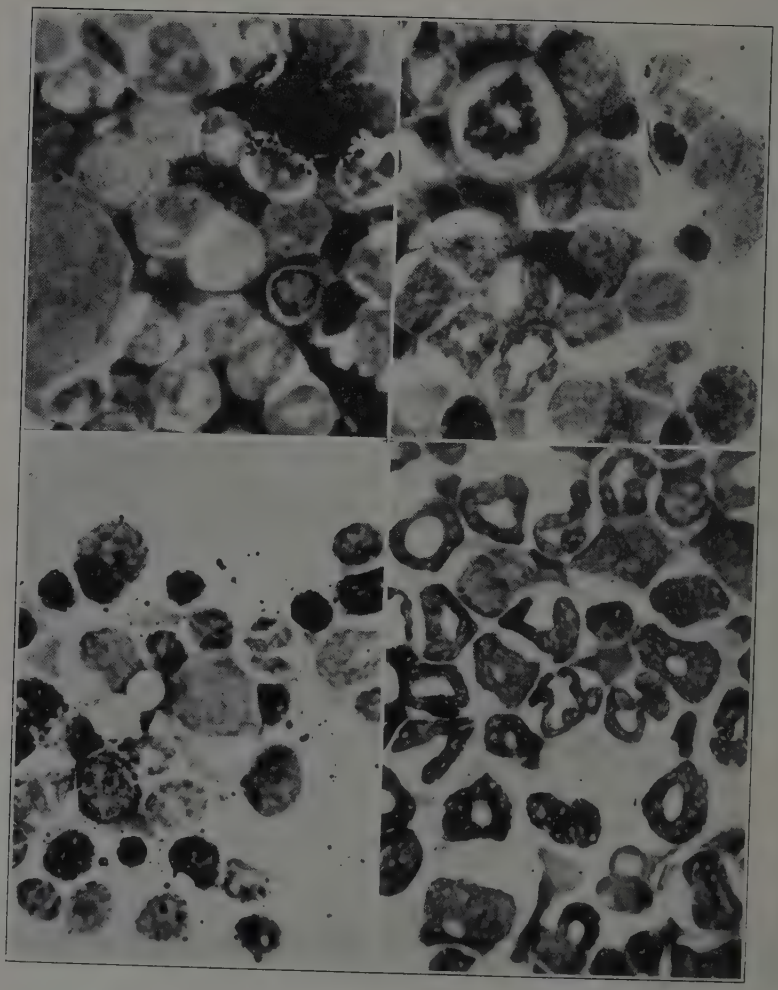

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Journal of Helminthology, Vol. XVII, *No.* 1, *January*, 1939, *pp.* 21-24.

On the Presence of Hooks on the Rostellum of *Hymenolepis microps*.

By Phyllis A. Clapham, Ph.D.

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Hymenolepis microps is a well known cestode inhabiting red grouse *Lagopus scoticus*, blackcock *Lyrurus tetrix* and capercailzie *Tetrao urogallus* in Europe. Recently the presence of this worm in America has been recorded by Jones and in Canada by Clark. In both cases the host was the ruffed grouse, *Bonasa umbellus*. It is a species which is easily recognisable because it possesses a very large cirrus sac in the mature part of the worm which passes unchanged into the gravid segments. It is found in grouse in the British Isles mainly in the summer months and then it occurs in enormous numbers. When the gut is opened the strobilae appear as a thick rope, being tangled so closely that it is very difficult to obtain a complete specimen.

The species has been known since 1850 when Diesing first described it. Since then it has been commented upon by Wolffhügel in 1900 and in 1909 Shipley devoted several pages of a paper to it. In spite of all this, however, the morphology of the scolex is very inadequately known as the worms are very fragile and break up readily. A break often seems to occur just behind the head, so that the scolex needs searching for. For some time it has been uncertain whether or not there are hooks on the rostellum. When the head has been mentioned, it has usually been described as being unarmed, but Shipley in his publication of 1909 announced the presence of hooks. From sections he estimated these structures as being at least 16μ long and described them as being like spines, closely packed in a single ring. They had a rounded end followed by a constriction after which they widened and then tapered to a fine point. These structures have never been seen since and they have received only scant recognition as they in no way conform to the typical hook of the genus *Hymenolepis*. This is roughly a T-shaped structure, with a short stem, the whole resembling the Greek letter λ.

A determined effort has been made in this laboratory to settle the question. A large quantity of preserved material has been examined from time to time, but no further information has been forthcoming. Recently however, there has been time and opportunity to examine some fresh material from a grouse. There were 19 scolices, 6 of which had the rostellum extruded. They remained in this state until death supervened. In this material it was possible to establish quite definitely the presence of a single ring of small hooks. It is easy to understand how they have been missed in the past, as they are very small and fall off quickly when the worm is fixed or when it is subjected to much handling and washing when collecting and separating from the faeces. It has unfortunately been impossible so far to obtain an entire specimen for mounting or for accurate counting. There seem to be about 20, but this number must be considered as only approximate and variation may occur. In shape, they fall into line with the other species of *Hymenolepis*. They measure 9μ long and consist of a slightly curved rod with a short branch measuring about 2μ attached at an angle near one end.

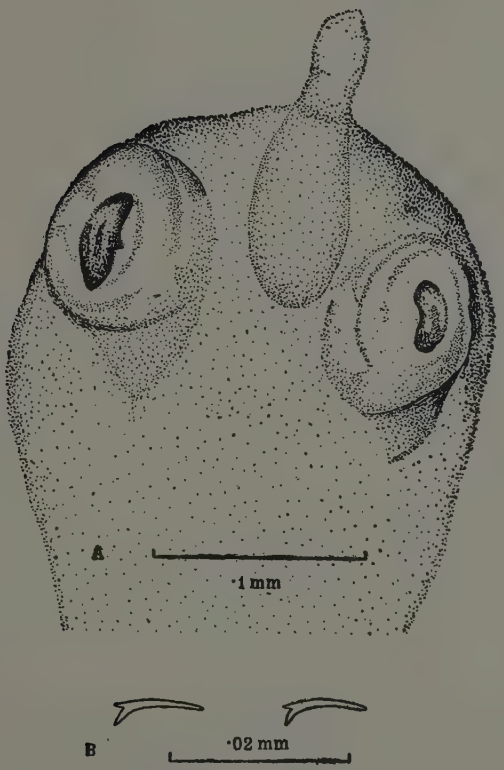
In other respects the scolex is not outstanding. It is semi-circular in lateral view with 4 muscular suckers. It varies but little in size, the measurements being 230μ–235μ across its widest part. The suckers, circular in shape, are about 80μ in diameter, varying between 77–86μ, according to the degree of contraction. There is a rostellum about 60μ long and 24μ wide when extruded. It can be retracted into a deep sac.

The spines as described by Shipley were carefully looked for, but nothing resembling them has been seen in either living or preserved material.

It is not suggested that this description of the hooks will be of much diagnostic value as they are too difficult to find and the large cirrus sac is characteristic of the species. But it is interesting to establish the presence of hooks which belong to the type characteristic of the genus. The spines described by Shipley were atypical and complicated any survey of the genus as a whole.

The classification and relationships of the various species of *Hymenolepis* have always been confused owing in part to the enormous size of the genus and in part to the inadequacy and inaccessibility of the species descriptions. Fuhrmann in 1906 in considering some bird species laid some stress on the number and shape of the hooks on the rostellum. He drew up and figured a chart illustrating the gradual development of the form of the hook

among the various species. Considered from this point of view, *Hymenolepis microps* comes into a group beginning with *H. anatina*, passes through such forms as *H. angulata* and finally reaches *H. fusus*. In all these forms the stem of the hook, *i.e.*, that part equivalent to the



A.—Head of *Hymenolepis microps*—lateral view.
B.—Two hooks from the rostellum.

upright of the letter T remains a definite structure and is attached at an angle at one end of the cross bar. It is a very common form within the genus and many of the species inhabiting the gallinaceous birds have hooks of this description.

But as Mayhew pointed out in 1925, too much reliance should not be placed upon either the number, the size or the shape of the hooks. For example, in the case of *H. macrostrobiloides* and *H. introversa*, the hooks are indistinguishable in size or shape, yet the species can be perfectly well differentiated on the nature of the rostellum which is a solid muscular organ in the one and can be retracted into a sac in the other. Mayhew therefore depends on the disposition of the genitalia and in his system the nearest connections of *H. microps* would be the following species—*armata, coronula, introversa, interrupta, macrostrobiloides* and *querquedula*—none of which are common species. He has constituted a new genus *Weinlandia* for the reception of these and other species. This method of classification has its disadvantages also as the relative position of ovary and tsetse is not constant throughout a single specimen. For instance in *H. phasianina* and other species where the three testes are arranged in the shape of a triangle, in the young part of the strobila, the arrangement is that of a right-angled triangle, the two antiporal testes being directly above one another: lower down, owing probably to pressure by the developing ovary and vitellaria the anterior testis gets pushed into other positions and it may come to lie in the middle line anterior to but between the other two testes or it may be pushed towards the antiporal edge of the segment.

The most easily seen character of this species is the large cirrus sac which is very muscular and in this respect the species is paralleled by *H. anatina, H. lanceolata, H. megalops* and others.

H. microps seems from the point of view of hook shape and position of the genitalia to be more closely related to those species which parasitize gallinaceous birds, *e.g., H. phasianina*, etc.

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On a New Amphistome Cercaria (Diplocotylea) from *Planorbis exustus*.

By J. J. C. Buckley, D.Sc.

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The cercaria species described herein was obtained from the Indian water snail *Planorbis exustus* in Assam. Only three of about fifty of the snails which were under observation in glass vessels were found infected, but these emitted many hundreds of the cercariae in the course of a few days, after which they were dissected for the purpose of studying the rediae and developing stages. The emergence of the cercariae was stimulated by placing the snails in direct sunlight, and after a free-swimming period of about an hour the cercariae readily encysted either on the sides of the glass near the surface of the water or preferably on lettuce leaves which were used as food by the snails. The snails had been collected late in December, 1934, from an isolated pool which, like many other tracts of water in the vicinity, was in the course of becoming dried up during the season of "cold weather."

Description of *Cercaria fraseri* sp. nov.

The body of the mature cercaria is about ·55 mm. long by ·45 mm. in breadth and the tail is about twice the length of the body (Fig. 3). The surface of the body is covered with what appear to be longitudinal striations, but in reality these are seen under high magnification to be minute rod-like pigment spots arranged in orderly rows. Internally, the body is heavily pigmented, especially in the region between the eye-spots, but it is less dense in the region of the oral sucker and acetabulum. There is a profuse supply of cystogenous cells which, together with the

pigmentation, make the body very opaque and tend to obscure the other internal structures. The mouth opening is unadorned by papillae and leads to an oral sucker ·08 mm. long by ·07 mm. in diameter, which is provided with two conspicuous pharyngeal pouches situated dorso-laterally. These measure ·05 mm. by ·05 mm. The oesophagus is thick-walled and lacks an oesophageal sphincter. Its length in relation to the caeca is seen in Fig. 4, which represents the body of a living cercaria considerably compressed between slide and cover-glass in order to expose the excretory and digestive systems. The oesophagus and caeca contain rectangular crystalline bodies. The acetabulum is very muscular and measures ·13 mm. in length by ·15 mm. in breadth.

The excretory system consists of a moderately large vesicle dorsal to the acetabulum and opens to the exterior by a conspicuous pore. Anteriorly two main branches run forward undulating slightly and about the level of the eye-spots suddenly become narrow, forming two horns which run between the eye-spots and are slightly expanded at their terminations. At the level of the junction of the oesophagus with the caeca a subsidiary branch runs directly posterior from each main branch and presumably has connection with the capillary system inside the acetabulum. The main branches contain spherical granules which are smallest in the posterior third of the vessels. No flame cells were observed. In the musculature of the acetabulum there is an excretory tubule which forms an almost complete loop, from the periphery of which smaller tubules are given off and these terminate in capillaries. In the tail of the cercaria there is a single tubule which in the posterior quarter expands to an elongated vesicle. External pores appear to be lacking from the tail.

The cyst (Fig. 5) is hemispherical, ·44 mm. in diameter and has a very thick wall of ·05 mm. Its surface is covered with residual cystogenous material containing rod-like granules.

The redia (Fig. 1) is comparatively small and contains either immature cercariae or daughter rediae. The cercariae are liberated from the redia in an immature condition and continue their development in the snail tissues. The redia lacks locomotor appendages of any kind. The birth pore is easily visible and is situated about ·15 mm. from the anterior end of the body. The pharynx is ·05 mm. in diameter and opens directly into the intestine which in the specimens examined was about a quarter the length of the body. The excretory system was not studied.

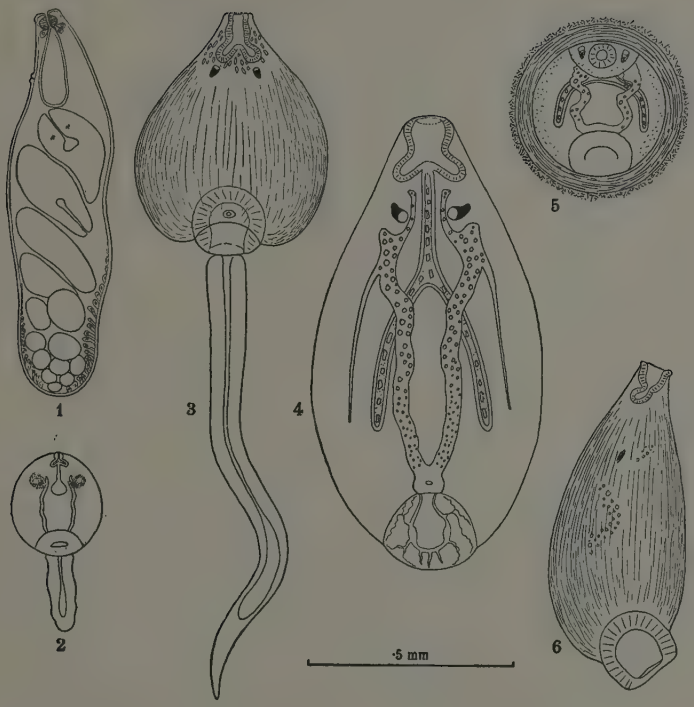

Fig. 1. Redia with immature cercariae. Fig. 2. Developing cercaria from snail tissue. Fig. 3. Mature cercaria, natural shape. Tail almost detached. Fig. 4. Body of mature cercaria, compressed to show excretory and digestive systems. Fig. 5. Cyst. Fig. 6. Artificially excysted cercaria, lateral view.

Relationships.

C. fraseri possesses nearly all the features which are characteristic of the Amphistome group Diplocotylea as defined by Sewell, 1922. In the absence of locomotor appendages in its redia, it shows a slight departure from the group; but this has already been observed in another member—*C. poconensis* Willey, 1930, and an emendation of this characteristic in the definition of the group would therefore appear desirable. At least eleven species of cercariae have been described in the literature,

which are referable to the Diplocotylea, and these are listed below together with their molluscan hosts.

Species.	Host.
C. diplocotylea Pagenstecher, 1857.	*Planorbis marginatus* et al.
C. sp. Looss, 1896.	*Cleopatra bulimoides* et al.
C. frondosa Cawston, 1918.	*Isodora tropica* et al.
C. inhabilis Cort, 1914.	*Planorbis trivolvis.*
C. diastropha Cort, 1914.	,, ,,
C. convoluta Faust, 1929.	,, ,,
C. missouriensis McCoy, 1929.	,, ,,
C. sp. Beaver, 1929.	,, ,,
C. poconensis Willey, 1930.	*Helisoma antrosa.*
C. indicae XXI Sewell, 1922.	*Planorbis exustus.*
C. kylasami Rao, 1932.	,, ,,
C. fraseri sp. nov.	,, ,,

C. fraseri is distinguished from the other species principally by the pattern of its excretory system and by the fact that its redia lacks appendages. Furthermore, it differs from the other two Indian species *C. indicae* XXI and *C. kylasami* in its well-defined muscular pharyngeal pouches. These organs appear to be somewhat inconspicuous in the latter two species.

The presence of pharyngeal pouches is one of the outstanding features of the Diplocotylea and in all probability relate this group with the important genera *Gastrodiscus* Leuckart, *Gastrodiscoides* Leiper and *Homalogaster* Poirier, in all three of which these organs are present and one species of which, viz., *Gastrodiscus aegyptiacus*, has been identified by Looss (1896) with a cercaria belonging to the Diplocotylea. Other species in the above list have also been identified with their adult forms. *C. diplocotylea* was shown by Looss (1892) to be the larva of *Diplodiscus subclavatus*. Beaver (1929) studied the development of *Allassostoma parvum* and concluded that both *C. inhabilis* and *C. convoluta* were identical with cercariae which, on being fed to bull-frogs and snapper turtles, developed into *A. parvum*. Willey (1937) has recently shown that *C. poconensis* is the larva of *Zygocotyle*.

It was thought that *C. fraseri* might be the larva of *Homalogaster paloniae* which was found about the same time post-mortem in three out

of five cattle from the locality where the infected snails were obtained. Cattle are very numerous in the district and the conditions necessary for infection of snails are present. Experimental feeding of the cysts to cattle was accordingly carried out.

Feeding experiments with Cysts.

The encysted cercariae were removed intact from the lettuce leaves or glass by means of a sharp cataract knife. They were sent to London where 350 were fed to each of two 2-months old calves by Professor Leiper at the Institute of Agricultural Parasitology, St. Albans, on the 21st February, 1938, about 62 days after they were collected. The faeces of these two calves was examined on six occasions up to seven months later but no amphistome eggs were found. The negative result obtained by this experiment was confirmed to a certain extent by a subsequent comparison of the morphology of the cercariae with that of living adult *H. paloniae*, whereby certain fundamental differences were revealed. Thus, the mouth opening of *C. fraseri* is quite plain, whereas in *H. paloniae* it is adorned with an elaborate frill composed of digitate papillae. There is an oesophageal sphincter in *H. paloniae*, a structure which is lacking in *C. fraseri*. In living specimens of *H. paloniae* compressed between two slides the main branches of the excretory system were made visible and these appear to unite in front of the anterior testis thus forming a transverse connection which of course is absent in *C. fraseri*.

Experimental feeding of 75 of the cysts to a pig in London about a month after encystment also proved negative, so that the possibility that the cercaria is the larva of *Gastrodiscoides hominis* was also practically ruled out. Furthermore, this worm appears to be absent in either pigs or man in the locality where the infected snails were found, although it is of course known to occur in other parts of Assam and was found commonly as a human infection by the writer in the Kamrup district in Upper Assam.

The possibility that *C. fraseri* might be the larva of *Gastrodiscus secundus*, which has been recorded in equines in Assam, was unfortunately not followed up. It is unlikely, however, that the cercaria of this species would differ as much in structure from the cercaria of the closely related species *G. aegyptiacus* described by Looss (1896) as the latter certainly differs from *C. fraseri*.

SUMMARY.

1. A new species of amphistome cercaria from *Planorbis exustus* in Cachar District, Assam, is described.

2. Feeding experiments with the encysted cercariae to two calves and a pig proved negative.

ACKNOWLEDGMENT.

The new species is named for Dr. G. Fraser, M.D., Ch.B., D.T.M. & H. (Eng.), Medical Superintendent, Labac Central Hospital, Cachar, Assam, to whom I am greatly indebted both for his hospitality and for the facilities made available to the writer at the hospital laboratory.

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Journal of Helminthology, Vol. XVII, *No.* 1, *January*, 1939, *pp.* 31-38.

Experiments on the Use of Isothiocyanates in the Control of the Potato Strain of *Heterodera schachtii* (Schmidt).

By Enid M. Smedley, M.Sc.

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Chemicals containing the isothiocyanate radical were first tested against the larvae of *Heterodera schachtii* during the summer of 1935. At that time a large number of chemicals was being tested under the guidance of Prof. R. T. Leiper, F.R.S. As a simple routine procedure, designed to eliminate chemicals unlikely to prove of any practical value, hatched larvae of the potato strain were placed in solutions of each chemical, varying in strength from 1% to ·01%, a proportion of the larvae being removed at the end of various periods to clean tap-water. By this means it was possible to determine whether the larvae were really killed or merely rendered immobile by their exposure to the chemical.

Three chemicals of the isothiocyanate group viz., phenyl, ethyl and n-butyl isothiocyanates were tested in this way. Although all three gave good results (·02% ethyl isothiocyanate kills in 2½ hours) they were not at the time regarded as of any unusual interest, particularly as their pungent odours render them unpleasant in use.

At the conclusion of these preliminary tests, the substances which had proved to be effective in killing the larvae were subjected to further testing against viable cysts taken from infected soil. Those which give off a vapour were first tested by exposing dry cysts to air saturated with the vapour in a closed jar for periods of from one to three days. The cysts were then removed, washed in clean air, soaked in tap-water and placed in fresh potato root leachings. By testing in this manner the three isothiocyanates referred to above, it was found that twenty-four hours' exposure to any one was sufficient to prevent hatching for a period of two

months. The cysts exposed to n-butyl isothiocyanate showed a slight amount of hatching during the second period of two months, and in this case 3 days' exposure was necessary to produce complete cessation of activities. In the case of phenyl isothiocyanate, a further more detailed test was performed, with exposures varying from 15 minutes to 24 hours. With exposures up to two hours there was no apparent difference in the hatching between the treated cysts and the untreated control, but from four to twenty-four hours' exposure produced complete inactivity for the first six weeks, followed by a very limited amount of hatching. The cysts exposed for twenty-four hours were quite dead. Apparently twenty-four hours is the critical exposure time for the vapour of this substance.

A similar test performed with p-hydroxyphenyl isothiocyanate showed that the cysts were quite unaffected by three days' exposure to the vapour, and subsequently hatched in fresh root excretion at the normal rate.

Very much more interest attaches to the results of another type of test, i.e., the action of very dilute solutions on the soaked cysts. Samples of one hundred cysts were placed in solutions of strengths varying from ·01% to ·0001% and records kept of the numbers of larvae emerging over a two months' period of continuous immersion in the chemical. Ethyl isothiocyanate in a dilution of ·004% inhibited hatching for a period of six weeks, larvae subsequently emerging at the normal rate. In the same concentration of n-butyl isothiocyanate, only 24 larvae hatched as compared with 300 in ·001%, and 8,500 in the control with fresh root excretion. o-Tolyl and p-tolyl isothiocyanates inhibited hatching at ·01%, but when the cysts were removed from the solutions into root excretion, they began to hatch freely. In phenyl isothiocyanate however, practically no hatching occurred at any of the strengths tested. Numerous repetitions of the experiment with very carefully prepared solutions, have shown conclusively that a concentration of ·001% (*i.e.*, one part in 100,000) of this substance is lethal to the cysts. Further tests in which a proportion of the cysts were removed from the solution into root excretion after short periods, have shown that practically no larvae will emerge from cysts which have been exposed for only three days to this high dilution, ·001%. Phenyl isothiocyanate thus appears to exert a selective action on the dormant stage of the parasite, its efficacy against the cyst being far greater than could be anticipated from the

earlier tests against the free larvae. A strength 20 times greater, ·02%, was required to kill the larvae in 7 hours, while they survived two days in ·004%. This is contrary to usual experience, the majority of chemicals being far more effective against the *Heterodera* larvae than against the cysts.

It was decided to lay out a field experiment at Potton, Bedfordshire, for the 1937 season. The first problem to be dealt with was the all-important one of preparing the chemical in a form suitable for incorporation with the soil, as in its natural form as a sticky oil it would be quite impossible to distribute evenly. This problem was satisfactorily met after some experimentation by the Research Department of Imperial Chemical Industries Limited (Dyestuffs Group). It was found possible to adsorb a small percentage of the oil on to talc dust, thus yielding a product sufficiently dry to be easily spread over the land, and without any tendency to form lumps in the soil.

On a small triangle of land of about one-fifth of an acre, a random block experiment was laid out, as shown in the accompanying plan. Each plot had an area of exactly 1/88th of an acre, so that each block was 1/22nd of an acre, and the entire plan 2/11th of an acre in extent.

The whole area was first rototilled several times. As the land had not been planted in 1936, this served the double purpose of even distribution of the cysts present and of cultivation. Three treatments were to be tested, viz., 2 cwt., 1 cwt., and $\frac{1}{4}$ cwt. per acre, each repeated four times, the remaining four untreated plots serving as controls. It will be understood that these amounts refer to phenyl isothiocyanate itself, much larger amounts of the dust being necessary.

Soil samples were taken by means of a steel cylinder, hinged for easy opening, 9 in. long by $1\frac{3}{4}$ in. in diameter. Six samples were taken from each plot, and the chemical was then applied and incorporated by twice rototilling. Three weeks later composite samples were taken for each treatment by mixing samples from each of the four plots of the treatment. The whole area was planted on that date, May 31st, with Majestic potatoes, so spaced that each plot received the same number of rows, each with the same number of potatoes. As no manuring had been done a 3 : 3 : 2 mixture of superphosphate, potash, and ammonia was applied on June 24th at the rate of 15 cwts. per acre along the rows.

Before the application of the artificials the plots which had received the 2 cwt. treatment were practically free of weeds, and the potatoes showed much better growth than those on the controls and lighter treatments. Subsequently this difference became gradually less noticeable. At the end of the growing season, however, the controls and lighter treatments died down noticeably earlier than the 2 cwt. plants. This observation is consistent with a lower incidence of "potato sickness" on the 2 cwt. plots.

Still further evidence of the reduction of the attack on the treated plots is given by cyst counts from the growing plants. One plant was removed with as much of its soil as could be kept intact from each of four plots representing the four treatments. In the laboratory the roots were carefully separated from the soil in each case, and the new cysts attached to the roots were counted. The plants were chosen completely at random, so that the counts cannot be said to be representative. They are very interesting however, and so are set out here :—

No.	Plot	Treatment	No. of new cysts
1	1	2 cwt.	74
2	13	1 cwt.	247
3	12	½ cwt.	578
4	4	Control	655

The Plate shows the actual cysts as they were collected in the laboratory.

The crop was lifted about the middle of October, the yield from each plot being kept separate. A very deep debt of gratitude is owing to Dr. R. H. Hurst, of the Institute of Agricultural Parasitology, for invaluable assistance with the statistical work involved in evaluating these results. The standard error and the yields for the four treatments in pounds and in tons per acre are set out in the table below :—

Yield	Treatment				Standard Error
	2 cwt.	1 cwt.	½ cwt.	Control	
Pounds per 4 plots ...	984·5	761	669·5	730	49·455 lbs. per acre
Tons per acre ...	9·7	7·5	6·6	7·2	0·49 tons per acre

1 cwt. (16)															
2 cwt. (5)			¼ cwt. (9)				¼ cwt. (12)				Control (14)				
Control (4)			1 cwt. (8)				2 cwt. (11)				1 cwt. (13)				2 cwt. (15)
Control (3)			Control (7)				¼ cwt. (10)								
1 cwt. (2)			¼ cwt. (6)												
2 cwt. (1)															

Fig. 1.—Phenyl isothiocyanate applied on Randomised Blocks in Field Experiment at Potton, Beds. Not strictly to scale.

Application of the " t " test shows a significant difference between the yields of the control and the 2 cwt. plots, the probability being less than one in a hundred. There is no experimental significance in the difference between the control and the 1 cwt. and ½ cwt. yields.

A short time after the lifting of the crop, the whole area was twice rototilled to distribute evenly throughout the soil the new cysts formed during the season of the experiment. Samples were then taken as before of the soil from each plot. The work of assaying the cyst content of the soil before and after the experiment was then undertaken. In the following table, the average counts for twenty 50 g. samples from each treatment (five from each of the four plots of the treatment) are shown as they were at the beginning and at the end of the test. In addition, the average counts of ten samples from each of the composite samples taken just before planting are included. Unfortunately these figures fail to prove anything, except that the cysts are not sufficiently well distributed for it to be possible to obtain reliable averages from such small numbers of samples.

	2 cwt.	1 cwt.	½ cwt.	Control
Beginning of experiment	126	139	110	132
Before planting	146	133	121	123
End of experiment ...	120	145	93	116

When it is realised that between the beginning of the experiment and the time of planting nothing was done which could affect in any way the real cyst content of the soil, it will become clear that no significance of any kind can be attached to the counts taken at the end of the experiment.

A pot experiment which was performed simultaneously serves to confirm and strengthen the results of the field experiment. Here it was possible to employ a much wider range of concentrations, from 40 cwt. to 1/64th cwt. per acre. Potton soil was used, and each strength was applied to two pots. One great advantage from the experimental standpoint is the much greater uniformity of distribution of the chemical obtainable in the pot experiment—an important point, as will appear presently. The pots were planted three weeks after treatment, and were grown in the open air, sunk in peat moss. After eight weeks' growth the

plants were tipped out, and the following cyst counts taken from the roots at the soil/pot surface :—

Control	1077 and 1380
1/64 cwt. per acre	995 „ 698
1/32 „ „ „	786 „ 1192
1/16 „ „ „	1375 „ 760
1/8 „ „ „	980 „ 721
1/4 „ „ „	873 „ 753
1/2 „ „ „	741 „ 809
1 „ „ „	107 „ 157
2 cwt. to 40 cwt.	no cysts

This very striking series serves to show how very strongly field treatment is hampered by the inadequacy of our present methods of incorporating chemicals with the soil. That there was in these pots very real control at 2 cwt. and over is borne out by hatching tests performed after the potatoes had ceased growing. 50 g. of soil was removed from one pot of each treatment from ½ cwt. to 10 cwt. and also from one of the controls. The cysts were washed from these samples, counted, and placed in fresh root excretion. The following table sets out the hatching results :—

	Treatment					
	10 cwt.	5 cwt.	2 cwt.	1 cwt.	½ cwt.	Control
No. of cysts in 50 g. sample	159	127	122	143	193	300
No. of larvae hatched in 8 weeks	0	0	2	59	89	145
Cysts dissected in fresh root excretion	dead	dead	dead	alive	alive	alive

It is clear that an application of 2 cwt. per acre is sufficient to give complete control of the parasite, when it can be brought into sufficiently close admixture with the soil.

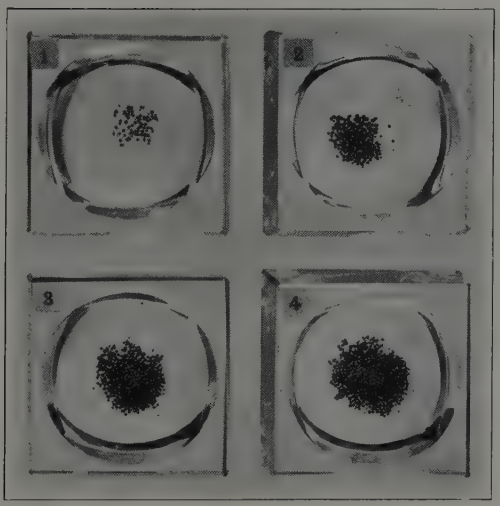
Summary.

1. It has been established that a concentration of ·001% of phenyl isothiocyanate is sufficient to kill the cysts of the potato strain of *Heterodera schachtii* when they are kept submersed in it for a period of three days or longer.

2. A randomised block field experiment has shown that there is a significant increase in the yield of potatoes grown in soil to which this chemical has been applied at the rate of 2 cwt. per acre.

3. It is demonstrated that cyst counts from soil samples taken from the field can be completely unreliable as an indication of the incidence of the disease, since the cysts may be very irregularly distributed in the soil.

4. Pot experiments and subsequent hatching tests have shown that the application of 2 cwt. per acre is lethal to the parasite when the chemical can be uniformly distributed throughout the whole volume of the soil.



New cysts collected from roots of potatoes grown on plots treated with phenyl isothiocyanate, Potton, 1937. (1) 2 cwt. per acre, (2) 1 cwt. per acre, (3) $\frac{1}{4}$ cwt. per acre, (4) control. (See Table on page 34).

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On the first outbreaks of Potato Eelworm (*Heterodera schachtii*, Schmidt) in Jersey.

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IN view of the spread of potato eelworm (*Heterodera schachtii*) in England and Scotland in recent years, it was decided in 1935 to examine farm soils in Jersey to ascertain if these were free from the pest. In three seasons, 1935 to 1937, 180 soils, the majority from fields which had grown potatoes for 10 or more consecutive years, were tested and all of them proved to be free from potato eelworm. These results, together with observations of numerous growing crops, indicated that the Island was substantially, if not entirely, free from the pest.

In Jersey, the farmers save their own seed of the local favourite early variety, *International Kidney*. Occasionally a little seed of promising new early varieties or of late varieties may be imported from England, Scotland or Ireland. To reduce the risk of importing potato eelworm on such seed potatoes Legislation was introduced in 1935, and from 1936 all potatoes entering the Island have been certified as free from *H. schachtii*. The Certificate is based on :

(a) examination of the land in which the potatoes were grown,
or (b) examination of the soil which dropped from the potatoes on riddling,
or (c) examination of the root system of the growing crop after 1st July in any year.

The first outbreak of potato eelworm was discovered in May 1938 in a private garden by Mr. Ing, Horticulturist, Experimental Station, Jersey. Four small areas were affected, of which two were planted with *International Kidney* and two with *Sharpe's Express*. The large numbers of cysts (some of which were empty) on the roots and in the soil indicated that the infection was a heavy one and was probably several years old.

The second outbreak was discovered on 5th August 1938, on a crop of outdoor tomatoes which were planted early in June after the early

potatoes had been lifted. Nearly all the tomato plants were apparently healthy and had reached the fourth truss, but in four places in the field the plants had almost failed to grow. Numerous rounded white and brown cysts were present on the roots and in the soil. Miss M. Franklin, Institute of Agricultural Parasitology, St. Albans, identified the cysts as those of *H. schachtii* and suggested the examination of volunteer potato plants growing among the tomatoes. This suggestion was acted upon and cysts were found on the roots of potatoes growing among the good and poor tomato plants. These observations, together with the examination of soil samples from several parts of the field, showed that the potato eelworm was present and that it was distributed throughout the field (4/9 acre). Cyst counts per 100 grams of air dried soils gave the following results :—

N.W. corner of field	(tomatoes	poor)	52 cysts.
S.E. „ „	(„	good)	23 „
N.E. „ „	(„	poor)	49 „
Middle of field	(„	good)	7 „

Twenty-one cysts were examined and no empty ones were found. The results indicate that the infection is a fairly heavy one and is probably recent, *i.e.*, about 4 to 5 years old. In 1933 or 1934, and again in 1937, imported seed of maincrop potatoes was planted in the field.

It remains to be seen to what extent potatoes and outdoor tomatoes will be affected by potato eelworm in Jersey, but the two outbreaks now recorded suggest that it may cause serious losses, in which case crop rotation would become necessary and this would lead to an appreciable reduction in the present acreage of potatoes and tomatoes and to a probable decrease in land values. At present no systematic rotation of crops is practised ; potatoes and tomatoes are usually grown year after year on the same land and rents of £9 per acre or more are quite common. The fact that farmers exchange seed among themselves would help to spread the pest rapidly throughout the Island. In an effort to localise the two outbreaks recorded the affected areas are being put down to grass.

Grateful acknowledgment is made to Professor R. T. Leiper, F.R.S. and Miss M. Franklin, Institute of Agricultural Parasitology, St. Albans, Herts, for help and advice on this problem.

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Field Experiments in Ayrshire on Control of *Heterodera schachtii* by the use of Chloroacetates.

By

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and

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The damage done by the potato eelworm to the early potato crop in Ayrshire has led to continued efforts to obtain a satisfactory method of control. The adoption of a rotation, as advocated by O'Brien & Prentice (1931) has resulted in a diminution of the loss, but this method is unattractive to the farmer as it means that he loses the use of land, as far as potatoes are concerned, for at least two years. Experiments on chemical control have therefore been carried out by this Department for a number of years, and in 1937, in an experiment to be described below, encouraging results were obtained with a hitherto untested compound—calcium chloroacetate ($Ca(CH_2ClCOO)_2$). As a result of this, a wide plan of experiments was drawn up for the following year of which three will be described below, along with a discussion of their bearing on the eelworm problem.

Various chemicals have hitherto been tested to determine their efficiency in controlling *Heterodera schachtii* and a complete account of the work to 1935 is contained in a paper by Hurst & Triffitt (1935). The work since that date has been unsatisfactory so far as definite results are concerned as the various findings have been highly contradictory even with the same substances. One of the few chemicals about which there is any unanimity is calcium cyanamide, as various workers (Edwards, Hurst, Franklin, Triffitt) report some degree of control by its use. In this case, however, the interpretation of the increased yields is confused

by reason of the high dosages used and the value of the cyanamide as a fertiliser. Edwards (1937), however, obtained a reduction in the cyst content of the soil by the use of cyanamide at over 3 tons per acre, and Hurst & Triffitt (1935) have shown that it will retard the emergence of larvae from the cysts, which would indicate that apart from its value as a fertiliser, calcium cyanamide has an ameliorating effect in cases of potato eelworm infection.

Material and Methods.

In the trials to be described potatoes of the variety Epicure were grown in Ayrshire with the co-operation of a number of farmers who kindly placed portions of their fields at the disposal of this Department for experimental purposes. The potatoes thus received the normal cultural and manurial treatment and were planted and lifted along with the rest of the crop.

The field plots were treated with various chemicals among which were, Ammonium chloroacetate, Calcium chloroacetate, Commercial calcium chloroacetate.

The experimental areas were divided into square plots each of 1/40th acre, the various treatments and controls being randomised and replicated. The chemicals, in powder form, were uniformly applied by hand after the field had been ploughed. Suitably calm weather was chosen for the applications as some of the compounds were very finely powdered and care had to be exercised to avoid drift.

Preliminary Experiment 1937.

This experiment was carried out at Jameston Farm, Maidens, Ayrshire. The soil was very sandy and the field heavily infected with *Heterodera schachtii*, having a cyst count of 300 per 50 grams of soil. An area of half an acre was devoted to the experiment and divided into twenty plots of which four were treated with calcium chloroacetate at the rate of 3 cwts. per acre, four at 5 cwts. per acre, eight were for a purpose outwith the experiment and four were left as controls. The plots were distributed as randomised blocks, and the compounds were applied on 3rd February. The potatoes were planted on 15th March, having been previously sprouted in boxes as is the usual Ayrshire practice.

By 7th June there was a marked difference in appearance between the various plots, the treated ones being in every way superior to the controls,

as the plants were taller, more spreading, and of a darker green than those grown on untreated soil (Fig. 1). By the end of June all the untreated plots were showing symptoms typical of eelworm attack.

Half of the crop was lifted on 5th July and the other half on 7th July, the produce from each plot being weighed separately. The results are shown in Table I.

TABLE I.

JAMESTON FARM EXPERIMENT, 1937.

Yield of ware in tons per acre.

Rate of application of Calcium chloroacetate.	Yield of Ware (tons per acre).
3 cwts. per acre	7·5
5 „ „	8·5
Control	4·8

It is apparent from the above table that applications of 3 or 5 cwts. per acre of calcium chloroacetate have resulted in a considerable increase in yield and as there is no apparent fertiliser value in this compound, it would appear that the increase in yield is due to the effect of the chemical on the incidence of the eelworm disease.

This contention is supported by the results of an examination of the roots of a large number of the plants while the crop was being lifted. In most cases the cysts were fully developed and were readily detached from the roots by shaking. It was not possible therefore to make an accurate count, but it was obvious that the number of cysts on the roots of the plants from the treated plots was much less than the number of cysts on the untreated plots.

So far as is known this 1937 experiment was the first occasion on which calcium chloroacetate had been used as a possible means of eelworm control.

1938 EXPERIMENTS.

(a) *Jameston Farm.*

In 1938 an experiment was carried out using the same plots at Jameston as in the experiment described above.

The calcium chloroacetate was applied on 16th February at the rates of 3, 5, and 7 cwts. per acre in the manner previously described. The potatoes were planted on 2nd March and notes taken from time to time on the growth and development of the plants in the various plots. On 29th April when the shoots were beginning to appear above ground emergence counts indicated that the chloroacetate had exerted a slight injurious effect on the growth of the sprouts as there were fewer plants visible in the treated plots. This was most marked in the 7 cwt. plots, but the depressive effect passed very quickly and was not apparent in later counts.

On 2nd June samples of the potato roots were collected from plants selected at random from the various plots. From these samples, lengths of about half an inch were removed so that the root tip was included thus assuring that the pieces were of approximately the same age and had been exposed to the eelworm infection for an equal period. These pieces of root were then stained in a solution of 2% Iodine in 95% Alcohol for twenty minutes, and de-stained in 95% Alcohol until the root tissues were colourless. After clearing and mounting in Clove Oil, the roots were examined microscopically for the presence of larvae, which, being much more retentive of the stain than the roots, could be seen in the tissue, stained a dark brown. Twenty root tips of each sample were examined and the number containing larvae noted. (See Table II.)

TABLE II.

JAMESTON FARM EXPERIMENT, 1938.

Yield of ware in tons per acre and Percentage of root tips invaded by Larvae.

Rate of application of calcium chloroacetate.	Percentage root tips invaded.	Yield of ware (tons per acre)
3 cwts. per acre	38	9·3
5 „ „	29	10·2
7 „ „	20	11·1
Control	67	6·6

It can be seen that the percentage of roots containing larvae decreases with increased dosage of chloroacetate. By 24th May there was a conspicuous difference in the plants, treated plots being darker green and

containing taller plants than the controls. In treated plots it was difficult to distinguish the individual drills when viewed from a distance of a few yards whereas a considerable area of soil was visible between the drills and between individual plants in the control plots.

The crop was lifted on 12th July and weighed. The results (see Table II) showed a marked increase in yield similar to that obtained in 1937 (see Table I), and the correlation of the yield and the invasion of the roots by larvae is striking. It was also noticeable that those plots which had been treated in 1937 and untreated in 1938 gave a higher yield than those untreated in both years, *i.e.*, that in 1938 there was a residual effect from the 1937 treatment.

(b) *Girvan Mains Farm.*

This was a much larger experiment than that described above as it consisted of eighty-one plots each of 1/40th acre. In this experiment randomised plots were treated with 2, 4, 6, and 8 cwts. per acre of calcium chloroacetate, some on 1st December, 1937, and others on 27th January, 1938. A series of dosage trials with commercial calcium chloroacetate at the rates of 2, 4, and 6 cwts. per acre were included in the scheme and applied on 11th February. Each treatment was in sextuplicate and there were nine controls.

The field was planted on 22nd February and there was a marked delay and in some cases a reduction in emergence, especially in those plots treated on 11th February. This reduction could be seen on all the plots treated on 27th January except where the rate was 2 cwts. per acre. Those plots treated with 8 cwts. per acre in December also showed a slight reduction in emergence.

The earlier treated plots caught up with and surpassed the controls by the beginning of May, but a late frost damaged the plants considerably, so that those on the treated plots did not get the benefit of the better foliage which they had developed. Owing to chemical injury plants treated on 11th February did not develop as well as the controls.

Roots were taken from this experiment also, and examined for invasion by larvae. In every case it was shown that a reduction in the percentage of invaded roots was obtained, although this was not so marked as in the case of Jameston Farm Experiment, 1938.

The crop was lifted on 21st to 23rd June and the results are expressed in Table III.

TABLE III.

GIRVAN MAINS FARM EXPERIMENT, 1938.

Yield of ware in tons per acre with reference to date of application.

Rate of Application of Calcium Chloroacetate	Applied 1st Dec., 1937	Applied 27th Jan., 1938	Applied* 11th Feb., 1938
2 cwts. per acre	7·0	6·8	3·4
4 „ „	7·4	5·4	2·6
6 „ „	7·3	5·6	2·3
8 „ „	6·8	3·1	—
Control		6·4	

It is obvious that the increase in crop in this experiment was not so great as in the Jameston Farm Experiment 1938, but a number of factors contributed to this. Of these, probably the most important was that the field had not been in potatoes the previous year, for it is generally accepted that eelworm attack is less severe in such cases. Furthermore the early digging of the crop (21st June) deprived the plants on the treated plots of the full benefit of their improved foliage.

(c) *Monktonhill Farm Experiment.*

This experiment was conducted at Monktonhill Farm, Troon, and consisted of forty-eight plots each of 1/40th acre. Here the compounds used were ammonium chloroacetate, calcium chloroacetate and commercial calcium chloroacetate at 3 and 6 cwts. per acre. Twenty-four of the plots were treated with calcium chloroacetate or commercial calcium chloroacetate on 9th March, and the other nine with ammonium chloroacetate on 14th March, five of these at the rate of 3 cwts. per acre and four at 6 cwts. per acre. All other treatments were in sextuplicate. The potatoes were planted on 24th March and the crop lifted on 23rd and 24th August.

On 20th June, when the plots were examined and given a numerical rating based on the appearance of the plants, the treated plots were in all cases superior to the controls. The weight of the resultant crop lifted on 23rd–24th August is given in Table IV.

* *Commercial* Calcium Chloroacetate.

TABLE IV.

MONKTONHILL FARM EXPERIMENT, 1938.

Yield of ware in tons per acre.

Treatment	Rate of Application	
	3 cwts. per acre	6 cwts. per acre
Calcium chloroacetate	14·1	14·0
Commercial calcium chloroacetate	14·6	13·7
Ammonium chloroacetate	14·4	14·4

Control : 12 tons per acre.

There is in this case an increase in crop obtained by the use of both calcium chloroacetate and ammonium chloroacetate, but as in the Girvan Mains Experiment this field had not grown potatoes the previous year. It is reasonable to suppose that here, too, not much damage was done by the eelworm, and therefore no great increase in crop might be expected to result from its control.

(d) *Marress Farm (Irvine) Experiment.*

This large scale trial was carried out by the farmer, and consisted of two half-acre plots, one of which was treated with calcium chloroacetate at the rate of 7 cwts. per acre on 7th February, and the other left as a control. The plots were planted on 14th March and in this case a Second Early variety, Doon Star, was used. By 1st June the difference in the plots was very apparent, the treated plot being very much better than the control.

The potatoes were dug on 13th to 15th August and the yields of ware were :—

Treated Plot ... ... 13·5 tons per acre.
Untreated Plot ... ... 3·2 „ „

At the time of digging, the plants on the treated plot were still green and growing, while the untreated plots were withered down to the ground (Fig. 2). This field had been in potatoes the previous year.

DISCUSSION.

From the experiments described above it appears that calcium and ammonium chloroacetate, although having a phytocidal action, have the property of increasing the yield of potatoes in a field infected with the potato eelworm, *Heterodera schachtii*.

The phytocidal action of calcium chloroacetate was especially marked on the potatoes in the Girvan Mains Experiment, but it was observed in all the other experiments, in that there was a marked reduction in the number of weeds growing in treated plots. This phytocidal effect passed off after a time, but in no case had it entirely disappeared three weeks after the chloroacetate had been applied to the soil. There is therefore a period after the treatment of the soil with these chloroacetates during which it is unsafe to plant potatoes. The main damage done is to the roots, as the tips are burned and growth delayed. In high concentrations, however, the sprouts are killed, hence the application of chloroacetates to the field should be done judiciously.

Calcium chloroacetate seems to have a beneficial effect on the plant apart altogether from the question of eelworm control; this can be seen not only in the darker green and more luxuriant growth of the potato plant, but in other experiments where calcium chloroacetate was applied on top of ryegrass, the grass showed this improvement.

Apart from the above action of calcium chloroacetate there is a very definite effect on the eelworm itself. This is manifest in a reduced number of infected roots in treated plots, and in the case of the Jameston 1938 Experiment this reduction could be easily detected even after three months' growth of the potatoes. In roots examined from other experiments the distinction was not so marked, especially where the treatment had been applied to the soil some months before planting. In these cases there was practically as much infection in the treated plots as in the controls.

It seems probable therefore that the chloroacetates affect the eggs in the cysts in such a manner that the escape of the larvae is reduced, or at any rate delayed, and this deduction has been borne out by experiments in watch-glasses. In these, the number of larvae escaping from cysts pretreated with a calcium chloroacetate solution was much less than that from untreated cysts and there was a considerable delay before any hatching began. Similar findings have been reported by Smedley (1938). Our experiments also indicated that at concentrations up to 1% calcium chloroacetate, the free larvae were not killed. This would account for the fact that even at the highest rates of application of calcium chloroacetate in the field experiments, there was always a small percentage of infection, due possibly to larvae which had overwintered in the soil (Franklin (1937)) and were able to cause immediate infection.

The action of chloroacetates in the field is therefore two-fold as there will be fewer larvae released from the cysts to cause infection of the potato roots and those larvae which do escape may not have sufficient time to complete their life-cycle before the crop is lifted. Few new cysts will therefore be added to the soil and thus the parasite may in time be eliminated.

The beneficial effects of calcium chloroacetate in the control of weeds should not be overlooked.

The authors wish to thank the Pest Control Research Committee of Imperial Chemical Industries, Ltd., who collaborated in the carrying out of these experiments, for a grant and for supplying the chemicals used in these experiments. They also wish to thank the Ayrshire Farmers, the Potato Marketing Board and the Agricultural Research Council for grants towards the cost of the investigation ; and finally the farmers who have so readily afforded facilities for the field trials.

A complete account of all the 1938 field experiments, hatching experiments, etc., will be published shortly.

Summary.

The effect of calcium and ammonium chloroacetates in increasing the yield of potatoes grown in Ayrshire fields infected with *Heterodera schachtii* is demonstrated.

From the examination of roots of treated and untreated plants it is believed that this increase in yield is due to a reduction in the eelworm infection, and from hatching experiments this reduction is thought to be the result of the action of calcium chloroacetate in retarding and reducing the hatching of larvae from the cysts.

The phytocidal action of the compounds is noted and it was found that a period of three weeks should elapse between treatment of the soil and planting of the crop if considerable damage to the sprouts and roots of the tubers is to be avoided.

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Fig. 1.—1937 Jameston Farm Experiment. Untreated plot (foreground), and treated calcium chloroacetate 5 cwts. per acre (background).

Fig. 2.—1938 Marress Farm Experiment. Untreated plot (left), and treated calcium chloroacetate 7 cwts. per acre (right).

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Field Tests on the Value of Calcium Chloro-acetate for controlling the Potato-sickness associated with the Root Eelworm, *Heterodera schachtii.*

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Field experiments were conducted by the writer during 1936 to test the value of certain chemical substances in controlling potato sickness. The results were published in this Journal (1937) and it was shown that of all the soil treatments tested, the use of calcium cyanamide proved the most successful in stimulating the potato crops to overcome the deleterious effects of this disease. The control of potato sickness by means of calcium cyanamide presents, however, a difficult problem in that this material, unless used in exceedingly heavy amounts, merely aggravates the proper utilization of infected land on account of building up an enormous eelworm population. Apart from being an uneconomical proposition for general application on a commercial scale, dressings of cyanamide that are sufficiently heavy to protect potato crops from the adverse effects of potato sickness and to simultaneously reduce the nematode infestation of the soil may lead to a subsequently unbalanced state of the land so treated in regard to the optimum ratios of the essential nutritive substances necessary to the satisfactory growth of plants. In view of these circumstances, it was decided to extend the field investigations in 1938 to include a test of the effect of dressings of calcium chloro-acetate. The present paper records the results obtained with this chemical substance.

Opportunity is here taken of expressing sincere gratitude to Messrs. C. R. Gregory and R. P. Thomas, County Horticultural Officials for the Counties of Glamorgan and Monmouth, respectively, for very kind assistance afforded in connection with the field work involved in these tests. Grateful thanks are also accorded to the Imperial Chemical Industries, Limited, for so kindly supplying the calcium chloro-acetate.

General Arrangement of the Experiments.

The experiments were conducted on allotments in five, widely separated, localities. Potatoes had been grown continuously for many years on all the allotments selected for the purpose and the land in each case was markedly and uniformly "potato-sick" throughout, judging by the pathological condition of the crops during 1935–37. The experimental allotments at all centres were divided into plots of an area of 630 square feet, separated from one another by a strip of ground, two feet wide, planted with cauliflowers. A dressing of artificial manure at the rate of 8 cwt. to the acre was given to all the plots, including the controls, a few days before planting with potatoes. It contained 8·0% nitrogen (N_2), 16·0% soluble phosphoric acid (P_2O_5), 16·0% potash and 5·5% insoluble phosphoric acid. The variety of potato used was "Great Scot," being certified Scotch seed, and well sprouted. The distances of planting were 30 inches between the rows and 15 inches between the "potato-sets" in the row. The planting took place on all plots at the five centres in the earlier part of the first week in May 1938, and the customary conditions and practices in the respective districts for potato growing were observed.

The calcium chloro-acetate was applied at all centres some six weeks before the potatoes were planted. It was evenly spread by hand and, directly after the application, mixed well with the surface soil by means of garden tools. The application was done personally by the writer to ensure uniformity of treatment for all plots. The weather was sunny and calm and the soil at each centre in perfect condition for the incorporation of the material. Throughout the interval between the treatment of the soil and planting of the potatoes, the weather remained unusually dry for the time of the year. The chemical substance was tested in amounts equivalent to 5 cwt. per acre at Dowlais West and Risca, and 3 cwt. per acre at Blaina, Dowlais East and Pontypool (see Tables I, II).

Observations were made at intervals on the plants in all plots, including the controls, at each centre to determine the effects of different treatments upon (a) growth and pathological condition of the haulms, (b) yield of crop and (c) nematode population of the soil as represented by cyst counts.

TABLE I.
Effect of Treatment on Yield of Crop.

Centre	Calcium Chloro-acetate per acre	Index of plot	Per Plot.			Per Acre.			
			Market-able	Chats	Aver. Yield *	Average Yield *		Increased Weight over Control	
			lb.	lb.	lb.	Tons.	Cwt.	Tons.	Cwt.
Blaina ...	Control ... (Untreated)	1a	40·5	36·5					
		1b	34·5	38·0	37·5	1	3·0		
	3 cwt. ...	2a	61·5	46·0					
		2b	69·5	56·5	65·5	2	0·2		17·2
Risca ...	Control ...	1a	42·5	30·0					
		1b	49·5	43·5	46·0	1	8·1		
	5 cwt. ...	3a	78·5	26·5					
		3b	89·0	28·0	83·5	2	11·2	1	3·1
Dowlais (West)	Control ...	1a	23·0	36·5					
		1b	18·0	47·0	20·5		12·6		
	5 cwt. ...	3a	81·5	63·5					
		3b	94·0	67·0	87·5	2	13·6	2	1·0
Dowlais (East)	Control ...	1a	51·0	46·0					
		1b	47·0	44·0	49·0	1	10·0		
	3 cwt. ...	2a	151·5	41·0					
		2b	140·0	46·0	145·5	4	9·2	2	19·2
Pontypool	Control ...	1a	115·0	56·5					
		1b	108·0	50·0	111·5	3	8·3		
	3 cwt. ...	2a	205·5	44·5					
		2b	214·5	57·5	210·0	6	8·6	3	0·3

* Excluding " Chats."

Effect of Treatments on Growth and Pathological Condition of the Haulms.

At all centres, the plants on the untreated plots showed at first a normal development, but later their rate of growth diminished and by the latter part of July had practically ceased. At this time, many of the plants had already died down and the others were also obviously in poor condition, all exhibiting the typical stunted appearance of " sick " plants, with deformed foliage and weak, spindly stems.

The plants on the calcium chloro-acetate plots, in general, developed slower than those on the controls and were somewhat inferior in appearance for some weeks in the early part of the season. These adverse effects upon growth and vigour of the plants are attributed to the toxic action of the material since parallel experiments in earthenware flower pots gave the same result. Further, these depressing influences in both the pots and field trials were in direct relation to the amount of chloro-acetate added to the soil, being more evident where the heavier dressings had been applied. After the initial check, a marked stimulation of growth became apparent on the treated plots and by mid-July the plants showed a fair, but by no means good, haulm. This stimulation in the rate of growth was also accompanied by healthier appearance of the plants compared with that of the controls. There could be no doubt, however, from the general condition of the haulms that the plants were at this stage rather seriously affected by potato-sickness although, in contrast with the control plots, pronounced manifestations had been absent heretofore.

At the end of July it was quite obvious that the crops, both on the control and treated plots, at all centres were far below the average for the districts. At Blaina and Risca (Table I), the crops were, from the practical point of view, almost complete failures not only on the control plots but also on the treated ones. The foliage had the usual symptoms of severe potato sickness and was so dwarfed that the drills were not nearly covered. The foliage on the controls at Dowlais West and Dowlais East was equally as bad but the treated plots at these centres possessed a distinctly better growth of haulms. Of all the treated plots, those at Pontypool were the best, but even there the amount of leafage was not up to standard.

All the haulms on the control plots died down in the early part of August, while those on the treated ones persisted at Blaina and Risca (Table I) into the latter half of August and at the remaining centres, into September. The explanation of the differences in the growth produced on the treated and control plots was not determined. Since it was desired to compare the entire yield of the potato crop from each plot, no attempt was made to remove plants in the early stages of growth to ascertain if the addition of the chloro-acetate to the soil had exerted some influence on the development of the eelworm.

TABLE II.

Effect of Treatments on the Cyst Content of the Soil.

Centre	Calcium Chloro-acetate per acre	Index of Plot	Crop Weight per Plot *	Viable Cysts per 25 grammes	
				Before Cropping	After Cropping
			lbs.	†	
Blaina	Control (untreated)	1a	77·0	104·3 ±2·4	94·1 ±2·8
		1b	72·5	99·2 ±1·9	98·5 ±3·2
	3 cwt. ...	2a	107·5	100·5 ±2·1	101·3 ±1·5
		2b	126·0	98·6 ±2·3	109·2 ±1·6
Risca	Control ...	1a	72·5	140·4 ±1·8	107·1 ±1·7
		1b	93·0	142·0 ±1·5	115·7 ±2·1
	5 cwt. ...	3a	105·0	145·5 ±3·1	129·8 ±1·9
		3b	117·0	141·9 ±2·9	125·7 ±2·5
Dowlais (West) ...	Control ...	1a	59·5	151·8 ±1·2	89·5 ±1·0
		1b	65·0	156·2 ±2·2	87·4 ±0·4
	5 cwt. ...	3a	145·0	151·7 ±2·4	163·7 ±1·1
		3b	161·0	154·2 ±2·7	172·6 ±1·8
Dowlais (East) ...	Control ...	1a	97·0	148·6 ±1·4	124·4 ±0·5
		1b	91·0	146·4 ±1·3	128·3 ±1·2
	3 cwt. ...	2a	192·5	150·1 ±0·4	185·5 ±0·4
		2b	186·0	146·0 ±0·9	179·1 ±0·7
Pontypool	Control ...	1a	171·5	116·9 ±1·6	173·2 ±2·4
		1b	158·0	119·6 ±1·1	169·9 ±1·2
	3 cwt. ...	2a	250·0	115·8 ±0·8	210·3 ±1·3
		2b	272·0	114·7 ±0·6	219·4 ±1·6

* Including " Chats."

† Throughout this Table the Standard Error is calculated according to the following formula :—

$$\text{S.E.} = \frac{\sigma}{\sqrt{n}}, \qquad \text{where } \sigma = \sqrt{\frac{S(\overline{x} - x)^2}{n-1}}$$

Effect of Treatments on Yield of Crop.

The entire crop of potatoes on all plots was lifted in the third week of September. At the time of lifting, the total produce from each plot was shaken on a riddle 1¼ inch mesh. The tubers too large to pass through were weighed as marketable and included both " ware " and " seed " potatoes. Those passing through the riddle were weighed separately and

regarded as " chats." The results of these operations are tabulated in Table I where it will be noted that the yields support the inferences drawn from observations recorded during the growing period, and that the degree of potato-sickness was of a high and uniform order throughout each area upon which the plots in the present experiments were laid, judging by the weight of tubers obtained at the different centres on the untreated ground (Plots 1a, 1b).

From the examination of the detailed figures for the yields in the 4th, 5th and 6th column of Table I, it is evident that the application of calcium chloro-acetate to the land six weeks previous to planting had succeeded at most centres, to some measure, in assisting the plants to overcome the harmful effects of potato sickness, indicated by the increase in weight of tubers on the treated plots. The degree of protection derived from the treatment varied at the different centres, the greatest benefit occurring at Pontypool and Dowlais East. The average weight of marketable potatoes on the treated plots was 210 lb. at the former and 145½ lb. at the latter, compared with 111½ lb. and 49 lb. on the controls, respectively. There was an increase in yield at each of these centres due to the treatment on an average of approximately 97 lb. per plot or 3 tons to the acre. The yields of crop obtained on the treated plots at the other three centres were decidedly less satisfactory, particularly at Blaina and Risca where on an average the increase in weight over that on the controls was only 28 lb. and 37 lb. per plot or about 17 cwt. and 23 cwt. per acre, respectively. The reason for these contradictory observations is probably connected with the marked variations in the type of soil at the different centres. The soils at Pontypool and Dowlais East were well drained, light loams, moderately rich in organic matter while those at Blaina and Risca were rather heavy, retentive and in a lower state of fertility. The soil at Dowlais West greatly differed from that at any other centre as it is largely composed of peat. It is unlikely that the limitation of the yields produced at the various centres was associated with insufficient food supply available for the potato crop since the cauliflowers planted between the different plots at each centre made excellent growth and reached full maturity.

Under the particular conditions of the present experiments, dressings of calcium chloro-acetate in amounts equivalent to 3 cwt. to the acre (Table I, Plots 2a, 2b), were, in general, more efficient than applications

of this material at the rate of 5 cwt. per acre (Plots 3a, 3b), as reflected by the weights of crop. It is difficult, however, to state how far the adverse effects produced by the chemical upon growth in the earlier stages of development of the plants might have influenced this final result.

Effect of Treatments on Cyst Content of the Soil.

The general technique adopted for determining the cyst population of the experimental plots was essentially the same as that described by the writer (1937) in connection with the investigations conducted in 1936. Both the treated and control plots at all centres were sampled for measurement of their cyst content on two occasions, the first in March, 1938, a few days prior to application of the calcium chloro-acetate, and the second at the end of October, some six weeks after harvesting the potato crops. At all centres, each plot was thoroughly dug and the soil mixed well to normal depth of cultivation between the times of lifting of the potatoes and taking of the soil samples. This procedure was considered advisable since cyst counts from soil samples collected immediately after harvesting are not always comparable with those from samples taken previous to cropping, due to uneven distribution in the soil of the newly formed cysts.

The cyst contents of the plots both before and after cropping are summarised in Table II, where it is apparent that the calcium chloro-acetate at the concentrations tested (Plots 2, 3) did not show any promise of application on a field scale to the problem of reducing the viable eelworm population of land affected by potato sickness. In all instances where the material was used, an actual increase took place in the cyst concentration of the soil. It must not be inferred from this data, however, that a correlation existed between the dressings of the chemical and the increases in the number of cysts, save indirectly inasmuch as the applications influenced the health of the potato plants. From a close analysis of the cyst census before and after cropping, together with a consideration of the effect of the dressings upon health of the plants, it is evident that the nematode infestation of the soil increased in proportion to the amount of foliage growth or, alternatively, to the amount of yield when that was greater than about 2 tons of marketable potatoes to the acre. The best growth of haulms and the highest weight of potatoes were produced on the treated plots (Nos. 2a, 2b) at Pontypool where also

the largest increase in the cyst population occurred. These plots showed after cropping (Table II, final column) an average cyst count of 214 cysts per 25 gm. of soil as against 115 cysts before treatment for the same weight of soil, an increase of 99 cysts on the original finding. The least change in the eelworm infection of the treated areas at all centres is found at Blaina, where the average rate of increase in the number of cysts on the two treated plots was rather less than 6 cysts per 25 gm. of soil. Of all the treated plots, these also gave the poorest yield of marketable potatoes (Table I), averaging only 65½ lb. per plot compared with 210 lb. from those which received the same treatment at Pontypool. A similar association also existed at the other centres between the alteration in cyst content and the vitality of the plants, the increases in the cyst infestation, in general, being on the treated plots in direct relation to the degree of freedom from manifestations of potato-sickness in the haulms (p. 54) and to the gain in weight of tubers (Table I).

In contrast to the treated plots, the cyst concentration of the soil in the controls (Plots 1a, 1b) at all centres, except at Pontypool, was decreased as a result of growing potatoes, the amount of reduction being, on the whole, inversely proportional to the yield of crop and to the original cyst count. At Pontypool, on the other hand, the controls showed a considerable increase in the number of cysts on cropping with potatoes and were in this respect comparable with all the treated plots. In retrospect, it may be stated that in no instance, under the conditions of the present experiments, was the cyst population directly affected by treatment of the soil with calcium chloro-acetate. In all cases the nematode infection of the soil, as reflected by cyst counts before and after cropping, increased with progressive gains in yield when that was more than about 2 tons of marketable potatoes to the acre and declined with successive diminution in weight of crop when that was less than this figure.

The only reference in literature to research on the treatment of soil with calcium chloro-acetate for the control of a plant parasitic nematode is to Smedley (1938) who included it amongst other chloro-acetates in laboratory tests. Its action was tested by the writer on cysts of the potato strain of *Heterodera schachtii*, being thoroughly mixed with 100 gm. samples of infested soil at the rate of 1 ton per acre and kept moist in a warm room for three weeks. In hatching tests carried out at the end

of this period, it was found that some larvae were liberated from the cysts even under these conditions when the material was intimately incorporated with the soil and given the desired amount of moisture. These results taken in conjunction with those obtained in the present investigation seem to provide definite evidence that exceedingly heavy applications of calcium chloro-acetate would be necessary to protect potato crops from potato sickness and simultaneously ridding infected land of the eelworm.

Summary.

Continuing the work on which a paper was published by the writer in the Journal of Helminthology, April, 1937, it was decided to concentrate on calcium chloro-acetate in the 1938 field experiments on soil treatment for the control of the potato sickness associated with *Heterodera schachtii*.

Tests were made at five different centres in the counties of Glamorgan and Monmouth with amounts equivalent to 3 cwt. and 5 cwt. to the acre.

Appreciable benefit was derived from both treatments at most centres in so far as growth of haulms and crop weights were concerned. In no case were those beneficial results accompanied, however, by reduction in the eelworm-cyst content of the soil.

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On Flies as Intermediate Hosts of *Syngamus trachea*.

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UNTIL 1934 the life history of *Syngamus trachea* was usually considered to be direct though Walker in 1886 and Waite in 1920 had both tentatively suggested that earthworms play a part in the dissemination of the parasite. In that year, however, I was able to show conclusively in this laboratory that the rôle they play in transmitting the nematode is a very important one, for by means of this annelid it was possible to effect with certainty heavy infections in various birds of domestic importance. The following year it was shown also that *Syngamus* from starlings can easily be transmitted to chickens by earthworms: this method apparently overcomes the resistance due to a different "host strain" such as Taylor had previously encountered when he tried to infect chickens directly with material obtained from starlings. In 1938 using earthworms as vectors chickens, pheasants and partridges were easily given definite infections with various other "strains" of *Syngamus*. Consideration of these results made it no longer possible to ignore the importance of earthworms in the life cycle of "Gapes."

Taylor in 1935 reported that snails and slugs also make efficient vectors and in the same paper, and again in 1938, showed that earthworms and snails form important reservoirs of infection, for the nematode larva can remain viable in these animals for periods of several years. *Syngamus trachea* therefore occupies an interesting position among helminths in that it can complete its life history with or without an intermediate host. The present writer's experience leads her to the belief that the cycle can be completed more easily with a vector than without one, using free eggs and larvae.

Recently I have been able to demonstrate experimentally that a number of dipterous insects can also act as carriers. A recent paper by Ford and others (1938) dealing with the feeding habits of partridges showed that they are entirely carnivorous for the first three weeks of their life;

the great bulk of the food consisting of insects while earthworms form a negligible part. As the young partridge frequently suffers from gapes the possibility of some insect acting as vector was immediately considered. Further, no one who has observed chickens can have failed to notice their efficiency in catching flies and the energy with which they pursue them and also their liking for the maggots. A batch of chickens will account for a very large number of them in the course of a day. These observations therefore led to the following experiment.

Gapeworm eggs were separated from the faeces of infected chickens. Others were dissected from the adult worms post-mortem. The eggs were cultivated to the infective stage in the laboratory and were then spread over pieces of rabbit flesh. Female flies of the species *Musca domestica* and *Lucilia sericata* were captured and imprisoned in the vessels containing the infected meat. They laid their eggs readily on this material—very readily if the meat were a little decomposed. After deposition of a sufficient number of eggs the flies were removed and the maggots allowed to hatch and develop. They were voracious feeders and swarmed all over the meat. An interesting observation was made in one dish where the *Syngamus* ova had come from worms, not faeces, and in which several pieces of worm tissue were left. The fly larvae seemed to have a predilection for these fragments and quickly ate them up. After three days the maggots were divided into two groups. Some were allowed to pupate and to metamorphose and the resulting flies were fed to chickens hatched six days earlier. The others were fed before pupation.

When samples from each group were dissected, it was found that the eggs had been taken up and were still alive. The larvae had found their way to the fat body surrounding the gut where they were lying loosely coiled apparently without any cyst. This condition contrasts with that found in the earthworm where a cyst is developed, but is probably due to the short time which elapsed between infection and examination as the cyst is almost certainly produced by the host and not by the parasite. The vitality of the maggot did not appear to be impaired by the presence of the parasite but infected adult flies tended to be somewhat sluggish and would normally be easy prey for birds in the natural state. Of 12 maggots and 12 flies so examined, all the maggots were infected, each with a single larva and 10 of the flies each again with a single larva. Feeding experiments to 12 chickens showed that most of the unexamined larvae and imagines must also have been infected for of 6 birds fed 20 maggots

each, all developed "gapes"—the number of pairs recovered post-mortem being 1, 3, 4, 4, 4 and 8. Of the half-dozen birds fed on 20 flies each, 5 became infected; there being 2, 3, 5, 5 and 7 pairs respectively in the tracheæ.

The experiments described above are only preliminary and the beginning of a large scale group but they prove definitely that some flies both in the larval and in the imaginal states may act as intermediate hosts for *Syngamus trachea*. The *Syngamus* larva can apparently withstand the physical and physiological changes which accompany metamorphosis and still remain infective to chickens.

Further experiments are now planned to determine the range of insect species involved and the part that they play in the spread of the parasite in the field.

The life cycle of *Syngamus trachea*, containing as it does so much variation and so many possibilities, suggests interesting trains of thought. The life cycle of this species would seem to be undergoing changes. The simple direct life history is the original one and the species is still capable of direct passage to another host but with the interpolation of facultative intermediate hosts a more complex and specialized method of propagation is arising.

Many kinds of invertebrates must ingest the ova accidentally with their food. Originally such ingested larvae probably died. In rare cases some were able to survive and in a few of these cases the new conditions proved advantageous to the young parasites. At this stage the invertebrate was merely a mechanical carrier but gradually the physiological requirements of the larvae changed to suit these new conditions. For in the body of a carrier the larva has become shut off from a variety of adverse environmental conditions and its chances of survival have therefore been increased. At the present time the physiology of the larva is so fluid that it finds favourable conditions for life in several, possibly many invertebrates. As time passes on a consensus of accidental factors will lead to greater specialization of intermediate host. Thus it may be that wild birds, feeding mainly on pasture and wood land, will be brought into contact chiefly with earthworms and they will come to be infected with a strain of *Syngamus trachea* which can only complete its life cycle by means of an annelid. Other birds in a more domestic environment which have easy access to dung, with its abundant insect fauna, will come to be definitive host to a strain of the same species needing an

arthropod vector. It is quite likely that in the course of many generations the present species *Syngamus trachea* will develop into a number of new varieties or even species each with their own individual vectors. A number of intermediate hosts which have been added accidentally to its economy may thus provide conditions favourable to the evolution of distinct species in different definitive hosts. It does not certainly follow however that this will happen for, at the present time, there tends to be a physiological difference between the parasites from the chicken and the starling. This is revealed by the fact that it is not an easy matter to parasitize chickens with worms of starling origin by direct passage yet by using an earthworm as intermediary the difficulties disappear and infection occurs readily. The physiological specialization has actually been removed by the passage through the annelid.

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The Treatment of Experimental Trichinosis in the Rat with Butolan.

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INTRODUCTION.

METHODS of treatment of trichinosis have been reviewed by van Someren (1938), and it was stated that no experimentally confirmed therapeutic measure had yet been found for the treatment of this disease by destruction of the nematodes in the body. In an attempt to discover a satisfactory chemotherapeutic measure, an experimental trial was made of an anthelminthic "Butolan" (Bayer), which has been shown to give very satisfactory results in cases of oxyuriasis, and in some ascariasis infections.

Butolan is the carbamic acid ester of p-oxy-diphenyl-methane, which is administered in tablet form *per os*. Butolan decomposes in alkaline medium in the intestines to p-oxy-diphenyl-methane (p-benzylphenol), a substance which has been shown by Schulemann (1920) to have very marked anthelminthic properties, being lethal to Oxyuris in 5 minutes in a dilution of 1 : 4000. About 25% of this p-benzylphenol is found in the urine after 24 hours, showing relatively quick absorption which probably precedes the splitting of the Butolan in the intestines.

Butolan is therefore similar in some respects to thymol, the anthelminthic action probably depending on the presence of phenolic hydroxyl, and since it is a substance which is absorbed and partly excreted in a relatively unchanged form, it seemed as if it might have a lethal action not only on adults, but also on larvae in the tissue fluids.

METHODS.

Nine adult and eleven halfgrown (90–120 days) white rats were infected with doses of 200–400 encysted Trichinella larvae, and thirteen of these treated at varying times after infection with varying doses of Butolan, the remainder being kept as controls. The tablets of Butolan were ground

and intimately mixed with the bread given to the rats; in general the drug was taken readily, but any refractoriness was overcome by a period of starvation before giving the medicated food.

Rats over 100 gm. in weight were given 0·25 gm. ($3\frac{3}{4}$ grs.) per day, which appeared to be the maximum dose which could be tolerated (in some cases for 21 days) without showing untoward toxic effects. In rats under 100 gm. this dose was rapidly toxic in 1–6 days, and the dosage was reduced to 0·125 gm. ($1\frac{3}{4}$ grs. approx.) per day, which was well tolerated.

For examination of the treated rats, the small intestine was slit open, the mucosa stripped and washed in warm saline, and direct counts of adults made, dead adults being readily recognised by their appearance and inactivity. In most cases where adults appeared to be killed by the drug, they were only slowly digested, the cuticle being passed in the faeces. Those rats which died during treatment were in most cases examined not more than 2–3 hours after death, but in rats 12 and 13 decomposition of the viscera had proceeded too far to enable intestine examinations to be made.

Where the rats were killed and examined under 30 days after infection, the larvae in the muscles were too young to withstand digestion, and the degree of infection was estimated only roughly. Rats killed at longer periods after infection were skinned, eviscerated, weighed and the carcases ground and digested in artificial gastric juice, the larvae being collected in a Baerman apparatus; the released larvae were counted by the dilution method and the result expressed as larvae per gm., since the rats in any one series of infections were of approximately equivalent weights. It will be noticed that in rats infected with 200 larvae only, the adults did not persist as long as three weeks in controls, as has been observed by McCoy (1932).

Results.

The results of the experiments are shown in the accompanying table. It is evident that Butolan has a curious variable action in individual rats, but it seems clear that total doses of 1·5 gm. or over result in a high mortality of adult Trichinella in the intestine with these infections, but evidently a small proportion of adults are not killed, resulting in a certain degree of muscle invasion, usually less than in controls. This may be due to adults reproducing in the caecum and colon which the Butolan possibly does not reach.

TABLE.

Results of treating adult (Nos. 1–9) and halfgrown (Nos. 10–20) trichinosed rats with Butolan (Bayer) *per os*.

No.	Inf. dose, larvae.	Treatment.			Days after inf. exam.	Living Trichinella recovered.	
		Days after inf. started.	Dose per day.	Total dose.		Adults.	Larvae.
1	4–500	7	0·25 gm.	1·0 gm.	14, killed	42	—
2	4–500	None	Control		15, killed	c.500	—
3	4–500	None	Control		22, killed	c.500	—
4	3–400	11	0·25 gm.	1·25 gm.	18, killed	1, dead	0
5	3–400	10	0·25 gm.	4.25 gm.	90, killed	0	32 per gm.
6	3–400	17	0·25 gm.	3·0 gm.	75, killed.	0	2 per gm.
7	3–400	None	Control		11, killed	c.300	—
8	3–400	None	Control		13, killed	210	—
9	3–400	None	Control		75, killed	0	60 per gm.
10	4–450	14	0·25 gm.	0·75 gm.	17, died	c.400	0
11	4–450	14	0·25 gm.	1·75 gm.	21, died.	30, dead.	Numerous.
12	4–450	15	0·25 gm.	1·5 gm.	21, died	?	Numerous.
13	4–450	21	0·25 gm.	1·5 gm.	27, died.	?	Numerous.
14	4–450	30	0·25 gm.	0·5 gm.	32, died.	c.250, many dead	Numerous
15	200	same day	0·25 gm.	0.75 gm.	3, died	20, 14 dead	0
16	200	same day	0·25 gm.	0·75 gm.	3, died	120, 50 dead	0
17	200	6	0·125 gm.	1·75 gm.	22, killed	0	Scanty.
18	200	6	0·125 gm.	2·0 gm.	45, killed	0	5 per gm. dead.
19	200	None	Control		22, killed	0	Scanty.
20	200	None	Control		45, killed	0	5 per gm.

It seems however that Butolan *per os* has no lethal effect on migrating larvae, though in the anomalous case of rat 18, where the larvae were estimated by direct counting in weighed portions of muscle, and which appeared to have as heavy an infection as the control, the larvae were all dead and being phagocyted in the muscles.

It is a curious fact that in rats 15 and 16 where treatment was started on the same day as infection, Butolan appeared to have a lethal action only when the larvae had reached maturity, and was evidently non-lethal to the immature larvae. This suggests the possibility that the larvae are in some way different from adults in their susceptibility to drugs, a supposition which appears likely also from previous experimental work (van Someren, 1938) showing that adult Trichinella are more easily destroyed than migrating or encysted larvae. That certain physiological differences do exist between adults and larvae has been shown by Bugge (1934); adult Trichinella are killed by artificial gastric juice, whereas encysted larvae naturally are not. On the other hand this resistance is shown only by encysted larvae and not migrating larvae or non-encysted larvae in the muscles. Nevertheless if such differences in susceptibility to drugs do exist, they would be an important consideration in the chemotherapy of the disease.

It seems therefore that the oral administration of Butolan might well be tried in early-diagnosed cases of trichinosis in an attempt to lessen the severity of the muscle invasion by destruction of mature reproductive adults, and since the latter appear to live for several weeks in man it would be advisable to try Butolan even in cases diagnosed late in the course of the disease.

In cases of oxyuriasis, the dosage recommended is 0·5 gm. (7½ grs.) three times a day for 7 days for adults. For children this dose is correspondingly reduced according to age and individual tolerance. This treatment might be tried in trichinosis or prolonged if necessary, and preferably followed by an aperient. Contra-indications would be evidence of severe kidney damage which might result in impaired excretion.

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Some Experiments on the Extra-Corporeal Hatching of the Eggs of *Ascaris suum.*

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Introduction.

Numerous attempts have been made in the past to induce the eggs of *Ascaris suum* to hatch outside the body of the host. Extra-corporeal hatching has been observed under a variety of conditions by different workers. Kondo (1920, 1922), Asada (1921) and others record hatching in water, charcoal and sand cultures. Wharton (1915) states that hatching will occur in alkaline digestive juices, while Martin (1913) records a similar phenomenon in pancreatic fluid. Many different explanations have been offered to explain this hatching. Wharton suggested that the interaction of algae and sand might have some effect. Ohba (1923), who found that hatching would occur in 0·2% hydrochloric acid and 0·2% sodium carbonate believed that extra-corporeal hatching was limited to very old cultures of eggs. Many workers are of the opinion that some stimulus normally present in the digestive tract is necessary for hatching.

McRae (1935), after a series of experiments, records the following. Negative results were recovered after exposure to 30°C. and 37°C. for one month, to artificial gastric juice (0·7%HC1+1% pepsin) for three weeks, as well as from alternate cooling and warming. Infective eggs kept in 2% formalin at room temperature never showed more than 1–2% hatching at any given time, while at the end of the time (two years) only 31% were still alive. Transfer of either newly-infective or old eggs from 0·2% hydrochloric acid to 0·5% sodium carbonate at 30°C. or 37°C. only induced about 2% hatching. She records evidence of spontaneous hatching. Positive results were obtained by stirring the eggs with moist sand. In one case, ten minutes of such treatment resulted in 80% of the eggs hatching. The larvae thus obtained are reported to have lived for only two days in Ringer's solution at 37°C. She expressed the opinion that many of the previous reports of extra-corporeal hatching of Ascaris

were misleading and that the setting-free of the larvae in these cases could not be interpreted as a biological phenomenon, but was due to external conditions causing injury to the egg-shell.

The experiments described in this paper represent an attempt to obtain some information regarding the factors governing the hatching of Ascaris eggs.

Technique.

Eggs, which are laid by the adult worm in the one-celled condition, were obtained by dissecting out the uterus from the adult worm and squeezing the eggs out of the lower end of it. The length of uterus used never exceeded 1–2 cms. since it was found that if longer lengths were taken, a large percentage of the eggs obtained did not develop. The technique used in rendering the eggs infective was that of Brown (1928) and consisted of incubating the eggs at 30°C. in water in Petri-dishes. On alternate days, carbon-dioxide-free air was passed through the liquid to ensure an adequate supply of oxygen for the eggs. An active larva was usually obtained in 16–20 days, at the end of which period the eggs were incubated for a further period of 21 days before being considered infective. The egg cultures were then stored in a cool shady place until required. In no case, except when specifically mentioned, were experiments performed on eggs which had been stored for more than six weeks.

A tissue culture technique was employed for experiments on the eggs, the hanging drop method being found particularly useful. In some cases, solid watch-glasses were used as culture dishes, the covers being sealed with vaseline to prevent evaporation of the medium.

Procedure.

In planning the experiments the following facts were considered :—

(1) The eggs hatch in the small intestine, and in all probability, hatching in that region is due either to the solution of the shell or to stimulation of the larva to break through the shell. Ransome & Foster (1919) suggested that hatching was due to active penetration of the shell by the larva inside.

(2) Intravenous injection of the eggs into the blood of the guinea-pig results in hatching of the eggs followed by the usual migrations of the larvae through the body (Ransome & Foster).

Consideration of these facts indicates that the causes underlying the emergence of the larvae probably fall under the following heads.

(1) If hatching is due to solution of the shell, then the exact nature of the solvent must depend on the composition of the shell, the solvent being probably either an acid, alkali or enzyme. The fact that the eggs hatch out in the blood stream very probably eliminates the digestive enzymes and acid as essential agents.

(2) If hatching is due to stimulation of the larvae then the stimulating agent must penetrate the egg-shell; this eliminates non-diffusible substances like proteins, etc. Very probable stimuli might be temperature, osmotic pressure or the presence of some inorganic salt.

The probable factors underlying hatching are therefore reduced to the following: temperature, osmotic pressure, H ion concentration or pH, presence of some inorganic salt or possibly an alkali effect.

It was suspected that the master factors were osmotic pressure and temperature; consequently the effect of Ringer's solution on the eggs was determined. Incubation of the eggs in Ringer's at 37°C. for 12 days did not induce hatching. Repetition of the experiment using Ringer's and also other salines ranging in osmotic pressure from that of a 0·2% sodium chloride solution to that of a 2·0% solution of the same salt, also gave negative results. It was therefore considered that in all probability factors other than osmotic pressure and temperature were involved in the process of hatching.

The effect of varying pH's was next determined, and a series of physiological salines was made up with pH's ranging from pH 6·0 to pH 8·0. No hatching was observed in any of these except in one saline of pH 7·4. In this solution a small percentage of the eggs had hatched out. An extra series was therefore made up with pH's ranging very closely around 7·4. No regular hatching occurred in any of these solutions although not infrequently it was found that a small number of eggs had hatched in some cultures. Too much importance should not be attached to these results as it was occasionally found that a small percentage of eggs in the reservoir egg cultures had hatched out spontaneously. Measurement of the pH of the experimental cultures at the end of each experiment showed that in those solutions whose pH lay between 6·8 and 7·7, there was no appreciable change in pH during the course of the experiment.

The effect of caustic soda at 37°C. on the eggs was next investigated. The solutions were made up in saline to strengths varying from 0·1% NaOH to 2·0% NaOH. All strengths of alkali of 1% or over rendered the larvae motionless inside the shells in less than two days. In no case was there any evidence of hatching despite much repetition, the shells never being dissolved. The 0·5–0·75% solutions appeared to have no effect on the shells and the larvae remained active for three or four days. Solutions of 0·4% caustic soda and under appeared to have no effect either on the larvae or on the shells. Alkali therefore appears to be ineffective in dissolving the shell.

Although it is considered unlikely that the digestive enzymes were necessary agents in inducing hatching, it was considered advisable to carry out a series of experiments on their action on the infective eggs, especially so, in view of the fact that previous workers claim positive results by using enzymes.

The effect of trypsin was determined by immersing the eggs in a solution of trypsin of the following constitution: 1% trypsin, 1% sodium carbonate. Eggs incubated in this solution at 37°C. showed no sign of hatching at the end of 14 days, although the larvae remained active throughout the whole of this period. Addition of acriflavein until its concentration was 1 : 1,000 in order to combat bacterial action did not in any way affect the result.

The effect of pancreatic juices was investigated by using a 1% solution of pancreatin in 1% sodium carbonate. A special extract of pancreas was also prepared by grinding up fresh sheep pancreas with sand, extracting with distilled water, precipitating the enzyme with absolute alcohol and redissolving in a 1% sodium carbonate solution. Negative results were obtained with these solutions even after ten days of treatment.

Bile obtained from the gall-bladders of pigs and of sheep as well as dilutions of it down to 1 : 30 aq. and 1 : 30 in Ringer's solution also failed to induce hatching. Negative results were also obtained with the dilutions of bile in trypsin and in pancreatin as well as in mixtures of these two enzymes.

The foregoing experiments indicate that intestinal digestion alone is insufficient to cause hatching. It was considered possible, however, that the process of digestion of the shell might possibly be a double one, the eggs undergoing a preliminary change in the stomach, the final

change involving solution of the shell occurring in the small intestine. To test out this possibility the eggs were submitted to the action of 1% pepsin in 1% hydrochloric acid for periods of six, twelve, twenty-four, thirty-six and forty-eight hours respectively followed by the solutions used in the last series of experiments. In all cases negative results were obtained.

McRae's experiments on the effect of agitation of the eggs with sand were also repeated. Shaking of the eggs with a suspension of sand in water, both by hand and mechanically, failed to induce hatching in more than about one to two per cent. of the eggs. Stirring the eggs with moist sand did cause rupture of the egg-shell and consequent liberation of the larvae in approximately ten to fifteen minutes. The liberated larvae were however found to be very badly damaged by the sand particles. It is considered probable that McRae's conclusion, that the hatching in this case is merely due to mechanical injury of the egg-shell and cannot be interpreted as a biological phenomenon, was amply justified.

It appears from the foregoing that none of the factors previously mentioned can be the main factor controlling the hatching of the eggs. It was felt that this factor might be the presence of some inorganic salt in the medium. As no suspicion was held regarding the nature of this salt, some attention was paid to the second ecdysis of Trichostrongylid larvae since this ecdysis involved the same change in the biology of the larvae as did the hatching in the case of Ascaris, viz., the change-over from a free-living to a parasitic mode of life. Lapage (1935) caused this ecdysis to occur by treating the infective larvae with hypochlorite in the form of the commercial preparation " Milton."

The effect of " Milton " and of a series of dilutions of it on the eggs was therefore determined, all experiments being conducted at 37°C. Concentrations of 1 : 4 and over did not result in hatching, the larvae inside the shells being killed in 24 hours or less. Some of the shells were dissolved away, but the larvae liberated were also dissolved away soon after they were liberated. Dilutions of 1 : 8–1 : 30 all resulted in some measure of hatching, the best results being obtained in dilutions of 1 : 15 and 1 : 20, in which solutions all the larvae hatched out in a period of eight to twelve days, about 50% being hatched out on the third or fourth days. Repetition of the experiment a number of times gave consistently similar results.

Careful observation disclosed that hatching was accompanied by the following phenomena :—

(1) The egg-shells showed slight distension which was less in the 1 : 15 solution than in the 1 : 20 solution despite the higher percentage of hatched larvae in the former solution.

(2) The larvae inside the shells were more active in the " Milton " solutions than they were in control cultures kept in Ringer's solution.

The egg-shells were thin and transparent and tended to lose their regular oval shape, presenting a very irregular swollen appearance.

These phenomena are, broadly speaking, the same in nature although not possibly in extent, as those which Lapage reports as accompanying the ecdysis of Trichostrongylid larvae in " Milton." He explains the action of this substance on the larval sheaths as follows: it causes a change to occur in the permeability and in the chemical composition of the sheath ; the former change results in an increase in the osmotic pressure inside, while the latter renders the sheath soluble in alkali, which is, of course present in " Milton." The change in the permeability and chemical composition, he attributed to the free chlorine present in " Milton." Experiments were therefore devised to ascertain whether or not this explanation was applicable to the hatching of Ascaris eggs under the same conditions. That the hatching was due to the hypochlorite complex, was proved by the fact that hatching occurred in solutions of sodium hypochlorite in the form of liqua sodae chlorinatae (B.P.) made up in saline to correspond to 1 : 15 and 1 : 20 dilutions of " Milton."

That the free alkali in " Milton " plays an important part, was shown by the fact that neutralizing the alkali with hydrochloric acid before making the dilutions resulted in only a very small degree of hatching on treatment of the eggs with it, even after ten or twelve days. Swelling of the shells, however, occurred to the same extent in this modified solution as it did in the ordinary " Milton " dilutions. It was considered that this swelling was due to a high pressure inside the shell, this being confirmed by the fact that gentle pressure of the treated eggs between slide and coverslip resulted in very violent bursting of the shell and vigorous ejection of the contained larvae, whereas similar treatment of ordinary eggs from a water culture resulted only in collapse of the egg-shell, the larvae having to escape from the ruptured shell by their own lashing movements. It therefore appears quite probable that the hypochlorite

complex does cause a change in the permeability of shell, resulting in an increase in the osmotic pressure inside.

An experiment was devised to determine the effect of the hypochlorite on the permeability of the shells in alkali. The eggs were treated with 1% chlorine water and 1 : 15 and 1 : 20 dilutions of " Milton " at 37°C. for 32 hours, after which they were immersed in different concentrations of caustic soda (1–0·1%). In all cases transfer to alkali resulted in some measure of hatching. Transfer to the more concentrated solutions resulted in the death of the larvae almost immediately after hatching. The more dilute solutions needed a greater time in which to act but hatching in those solutions was more even and more complete, about 40 per cent. of the larvae hatching in the 0·25% solution in 24 hours. Eggs treated with neutralised " Milton " hatched more readily on transfer to alkali than did those which had been treated with chlorine water. This is understandable when we consider the changes involved in the transfer ; the change in osmotic pressure on transfer from " Milton " to alkali was less than the corresponding change from chlorine water to alkali, and as this change was in a direction which tended to decrease the osmotic pressure inside, then, the smaller that change, the more favourable will be the conditions for hatching.

It appears from these experiments that the action of " Milton " on Ascaris eggs was the same as the action reported by Lapage for the same substance on Trichostrongylid larvae. Lapage regarded the changes in the sheaths as being specifically due to the free chlorine present in " Milton " or other hypochlorite solution used. It is difficult to understand how differentiation between free chlorine and hypochlorous acid is possible since it is an invariable rule that where free chlorine is present in aqueous solution, hypochlorous acid is present as well, and it is impossible to remove it completely. There is a considerable quantity of the latter substance present in chlorine water, since the chlorine present is partially hydrolysed, e.g., the chlorine in a 0·35% aqueous solution at room temperature is 32% hydrolysed, increase in temperature will increase the degree of hydrolysis. The degree of hydrolysis can be reduced by adding to the chlorine water or hypochlorite solution, quantities of hydrochloric acid. This reduction in the quantity of hypochlorous acid would, however, only be practicable to half its original concentration, since the ratio of the fall in concentration of the hypochlorous acid to the amount of hydrochloric acid added, would fall consistently with the addition of more and

more hydrochloric acid, until eventually a state would be reached when the addition of very large quantities of the latter substance would result only in very small decreases in the concentration of hypochlorous acid.

Differentiation between chlorine and hypochlorous acid does not therefore appear to have been attained in Lapage's experiments. An experiment was therefore devised with the object of obtaining a rough estimate of the values of the two substances as agents capable of inducing the aforesaid changes in the shells. The experiment consisted of adding to samples of 1% chlorine water, different amounts of hydrochloric acid so that the concentration of the added acid attained values ranging from 0·2 to 4·0%. This added acid drove back the equilibrium of the system towards the chlorine side of the reaction, so that the series would really be one for varying quantities of hypochlorous acid, the minimum quantity of the latter being present in the solution with the most added acid. Each solution was diluted with distilled water to a dilution of 1 : 15. Eggs treated with these solutions for times varying from 3 to 96 hours were then immersed in 0·25% caustic soda. The readiness with which eggs from different solutions of the series hatched in the caustic soda might then give a measure of the relative powers of chlorine and hypochlorous acid respectively as agents responsible for bringing about the changes in the shells. Despite every care, however, in the conduct of the experiment, there was no correlation between the amount of added acid and ease of hatching in caustic soda, and this experiment therefore failed to differentiate between the action of chlorine and hypochlorous acid.

Consideration of the constitution of " Milton " is very interesting ; it is alkaline in reaction due to the hydrolysis of the sodium hypochlorite in it. It can therefore contain no free acid and consequently all the chlorine present in it will be completely hydrolysed. It is thus impossible for " Milton " to contain any free chlorine. This conclusion can be confirmed by chemical tests for free chlorine. If potassium iodide and starch be added to a solution of " Milton " then no blue colour is obtained. A similar negative result can be obtained with a 1 : 15 or 1 : 20 dilution of " Milton."

The foregoing experiments and considerations, while not by any means conclusive, seem to indicate that the action of " Milton " is due to the hypochlorite present rather than to free chlorine. Experiments were therefore performed in order to determine the nature of the reaction involved. It was considered that the action might possibly be oxidative

in nature and the action of different oxidising agents on the eggs was therefore determined. Solutions of hydrogen peroxide of strengths ranging from 20 to 0·05 volumes available oxygen all gave negative results when used alone or when made up in a 0·75% saline. Addition of alkali to correspond to the alkalinity of " Milton " did not affect the result. Alkaline permanganate also gave negative results, although too much importance should not be attached to the action of this chemical since it appeared to have a harmful effect on the eggs, due possibly to the toxic effect of manganese. Similar considerations apply to negative results obtained with bichromate solutions. Solutions of sodium and potassium chlorate also gave negative results. The use of a number of other oxidising agents was precluded by the living nature of the eggs. Bubbling oxygen continuously through the infective cultures also failed to induce hatching. No support is therefore offered by the above experiments to the theory that the action of " Milton " is oxidative.

In order to obtain further information, certain other substances used by Lapage to induce the ecdysis of Trichostrongylid larvae were used on the infective Ascaris eggs. Infusions of garlic which gave positive results with Trichostrongylid larvae, did not induce any measure of hatching of the Ascaris eggs. A 1% solution of sodium sulphide caused hatching which was well advanced on the third day; the phenomena observed were the same as those observed when hatching occurred in hypochlorite solutions, except that the larvae did not appear to be stimulated. It was noticed that hatching in sodium sulphide was not by any means a regular phenomenon and when it did occur it was not nearly so well marked as it was in " Milton." Neutralisation of the free alkali prevented hatching, although on subsequent transfer to alkali hatching occurred as it did on transfer from neutralised hypochlorite to alkali. It appears from this that hatching in sodium sulphide is governed by the same factors as it is in " Milton." Lapage records a difference between the action of the two substances on the sheaths of Trichostrongylid larvae; whereas treatment of the shells with neutralised hypochlorites renders them soluble in alkali, this is not the case with sulphides, some other agent being necessary with this chemical. Treatment of Ascaris eggs on the other hand with either, renders the shell soluble.

It is thus seen that two distinct and dissimilar ions can play an identical rôle in modifying the shell of Ascaris, viz., the S ion and the C10 ion. Owing to the very different chemical nature of the two ions it is very

unlikely that the process is simply chemical, it is much more likely to be physical, probably adsorbtive, the ions being adsorbed by the egg shells, and altering their permeability in the same way as ions adsorbed from the soil alter the permeability of the membranes of the root-hairs of plants. This would account for the high internal pressure inside the shells. It is also conceivable that the adsorbed ions might catalyse the hydrolysis of the shells in alkali. That hypochlorite was removed from the solutions containing eggs was proved by micro-chemical analysis of solutions which contained eggs for a period of 48 hours; it was found that the hypochlorite content of these solutions was significantly lower than that of solutions without eggs at the end of a similar time. Such a result, however, was consistent with the removal of hypochlorite either chemically or by adsorbtion. More significant is the fact that the pH of the solutions containing the eggs falls during the course of the experiment to a greater degree than does the pH of the controls. Each of the figures given below is the mean of forty to fifty consistent readings.

Fall in pH of controls	0·02
Fall in pH of solutions containing eggs	0·06

These results are consistent with the removal of C10 ions by adsorbtion, since the removal of C10 ions by adsorbtion would increase the hydrolysis of the hypochlorous acid, setting free more H ion which would combine with some of the excess OH ion present, reducing the alkalinity of the solution, i.e., decreasing the pH.

The foregoing experiments appear to offer strong confirmation to the theory that the C10 ion is adsorbed by the shell. The factors governing the hatching of Ascaris in hypochlorite solutions may therefore be summarised as follows: certain ions such as C10 and S can be adsorbed by the egg-shell from solution. These ions alter the permeability of the shell and catalyse its hydrolysis in alkali. The change in permeability results in an increase in the osmotic pressure inside the shell and the catalytic action results in the solution of the shell and the consequent liberation of the larva.

In so far as it is difficult to determine to what extent the process of hatching in hypochlorite is paralleled by the process in nature, it is not possible to correlate with any certainty the behaviour of the eggs in hypochlorites with their behaviour in the alimentary tract. It appears to be more than a possibility that the action of " Milton " on the eggs is

not a biological effect at all, but merely a physico-chemical action resulting in a mechanical solution of the egg-shell. This is to a certain extent confirmed by the fact that the action of hypochlorites on the larvae is undoubtedly destructive and the aim in making up a solution in order to induce hatching was to obtain the minimum concentration which would induce efficient hatching so that the destructive action should be as small as possible.

If, however, the suggestion is accepted that the process of hatching in " Milton " is paralleled by the process in nature, then it remains to determine what are the natural factors which cause the changes in the egg-shell, since it is obviously very unlikely that the eggs should normally come into contact with hypochlorites. Lapage's suggestion that the Cl ion of the acid gastric juice may have the same effect on the shells as the free chlorine of " Milton " does not appear to be feasible, since it is very unlikely that the free chlorine molecule can have the same effect as the Cl ion, the latter being an electrically charged body with vastly different properties from the former, which in aqueous solution is merely one of the components of a reversible chemical system, in equilibrium with other components. In view, however, of the fact that experiments previously described in this paper indicate the improbability of chlorine being the active constituent of hypochlorite solutions as far as the hatching of Ascaris is concerned, it is unlikely that the question of the action of free chlorine being similar to that of the Cl ion of hydrochloric acid, need be considered. The question, however, remains, can the Cl ion of gastric juice have the same effect on the eggs as does the ClO ion of " Milton " ? That the answer should be in the positive seems unlikely, otherwise one would expect a simple saline solution as used in the earlier experiments described in this paper, to be able to modify the sheaths, since it obviously contains a sufficiency of chlorine ions. In any case the experiments in which the eggs were subjected to gastric digestion followed by tryptic digestion should, if the Cl ion were active, result in hatching, since the eggs on exposure to gastric digestion were exposed to the action of Cl ion in acid solution to be followed later by exposure to alkaline conditions in the trypsin solutions used. One is therefore led to the conclusion that as far as Ascaris eggs are concerned, the action of the ClO ion on the eggs is quite different from the action of the Cl ion, the former inducing the changes mentioned earlier, the latter being incapable of inducing these changes.

In view of the inability of the Cl ion to cause the modifications in the egg-shells we are forced to look elsewhere for the agent responsible. These agents might possibly be numerous and varied in character. It is considered possible that external influences may play some part in modifying the shells so that they hatch in the alkaline medium of the intestine. It is conceivable that ions other than hypochlorite and sulphide ions can have a similar effect on the egg-shell and the eggs may come into contact with these ions during their existence outside the host. The nature and source of these ions is problematic. They may be produced by bacterial action or possibly by the larvae themselves. The former of these suggestions is prompted by the fact that eggs from old cultures hatch out more easily than do eggs from younger cultures especially if there is much organic matter present in a decaying condition. In one case a culture was made up in water which contained a large amount of faecal matter. After 40 days the eggs were infective and the culture putrid, swarming with bacteria. This was kept for a period at 37°C. and examined frequently, Spontaneous hatching occurred all the time until at the end of eight weeks all the eggs present had hatched. The solution at the end of this period was markedly acid (pH 6·1). It appears that the bacteria present must have excreted some substance which modified the shells.

That the eggs themselves may be able to produce similar substances is suggested by the fact that eggs dissected out aseptically from the adult worm and incubated in sterile water at 28°C. until they became infective, and for a further six weeks, hatched out on transfer to 0·5% caustic soda to a small extent (2–3%). Periodic tests with sterile broth confirmed the sterility of the culture. It seems probable from this that bacterial action having been eliminated, the eggs themselves must have produced some substance which modified the shells.

The study of the behaviour of the eggs in sodium sulphide solution points to what might be a fruitful line of research. It is a known fact that hydrogen sulphide can under certain circumstances be produced in the small intestine as a result of bacterial action on sulphur-containing amino-acids. This may then cause the changes in the shells as it does *in vitro*. Results might also be obtained by inoculating intestinal contents, which have been centrifuged and filtered through a Berkfeld filter, with different strains of sulphur-producing bacteria.

No attempt was made to repeat the work of Yoshida and Toyoda (1938) as their work only came to the author's notice when this paper was almost ready for the press.

Conclusions.

1. Temperature, osmotic pressure, *p*H, and alkali are not in themselves sufficient to cause hatching of the eggs of *Ascaris suum*.

2. The digestive enzymes are also incapable of inducing hatching, at least *in vitro*.

3. Agitation of the eggs cannot be considered as a biological method of inducing hatching, and it is therefore improbable that they throw any light on the process.

4. As far as can be determined, hatching in hypochlorites is a complex process, involving a change in the permeability of the shell and also an increase in its solubility in alkali.

5. Sodium sulphide and sodium hypochlorite can induce hatching, being capable of bringing about the changes mentioned. They probably do so by being adsorbed on the egg-shell. The adsorbed ions alter the permeability of the shell and catalyse its hydrolysis in alkali.

6. It is difficult to determine to what extent the action of "Milton" is paralleled by the process of hatching in nature. It is felt that the possibility of the action of this substance being merely a physico-chemical solution of the egg-shell should not be overlooked.

7. It seems improbable that the place of the hypochlorite ion of "Milton" can be taken by the Cl ion in gastric juice, at least as far as the eggs of Ascaris are concerned, even if we do accept the suggestion that hatching in "Milton" is paralleled by the natural process.

8. If the action of "Milton" is similar to the natural phenomenon, then in all probability the changes in the egg-shell can be caused by the adsorption of some ion other than ClO or S. These ions may be produced by bacterial action, or by the larvae themselves.

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On the Presence of a Buccal Stylet in Adult Trichinella, and the Mode of Feeding of the Adults.

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The Oesophagus.

The peculiar structure of the oesophagus of the Trichuroidea was considered by earlier writers to be so unique amongst parasitic nematodes that for some time the trichuroids, and the mermithoids, were grouped in a separate sub-order, the Trichosyringata, characterised by the presence of a " capillary " oesophagus consisting of a row of cells pierced by a delicate intracellular tube. Studies by Chitwood (1931, 1935, 1937) however, have shown that the trichuroid oesophagus is in no way fundamentally different from that of other nematodes, the characteristic appearance being due to the serial reduplication of the sub-ventral oesophageal glands, forming the " cell-body " or stichosome, in which the posterior part of the oesophagus proper is embedded. The oesophageal glands thus come to lie outside the contour of the oesophagus, as in Contracaecum, Aphelenchus or Onchium.

The Trichuroidea and the Mermithoidea are now grouped as superfamilies of the sub-order Dorylaimina (order Aphasmidia) (Chitwood, 1937), which is characterised, among other features, by the presence of a buccal stylet (onchiostyle) at least in the larval stages.

Hoyberg (1907) first suggested that the larvae of Trichinella had a " boring apparatus," and Fülleborn (1920, 1923) showed that the larvae of *Trichocephalus trichiurus*, *T. leporis*, a Trichocephalus sp. from monkeys, *Trichinella spiralis*, *Capillaria hepatica*, and *Trichosomoides crassicauda* all possessed a distinct onchiostyle. Though it has been customary to assume that this larval stylet disappears in the adult stages of these forms, Li (1933) has described a distinct functional stylet in adult *Trichocephalus trichiurus* and in a Trichocephalus sp. from the macaque, and Chitwood & Chitwood (1937) state that one has been seen in adult *T. vulpis*.

It is now evident from the present study that the larval stylet also persists in adult *Trichinella spiralis*, and is functional; a fact lending further support to the close relationships of the Trichinellidae and Trichuridae, and the possible derivation of these forms from free-living Dorylaimoids.

The materials used in this study were six- and seven-day old adults obtained from experimentally infected golden hamsters, *Cricetus* (*Mesocricetus*) *auratus*, rodents which appear readily susceptible to trichinosis. Adult female Trichinella at this stage of infection in the hamster affords very favourable material for study of the anatomy, since the larvae in the uterus are not yet fully developed and do not distort the organs in the body cavity. The morphology of the oesophagus is essentially as Chitwood (1931, 1935) has described it, but some additional features must be noted.

The Buccal Capsule and Stylet.

The precise morphology of the capsule is very difficult to determine in living adults, and the stylet cannot be seen in living adults or in adults killed and preserved in the usual manner in hot alcohol. The relationships of these parts, however, can clearly be determined in adults which have been immersed alive in dilute neutral red (0·01% sol. in water) for two or three hours at 37°C. and then killed by addition of cold 70% alcohol. The structures are not stained by this method since the stain diffuses into the alcohol, but such treatment results in the worms being killed with the cephalic region greatly contracted, this resulting in marked protrusion of the onchiostyle, which may be seen distinctly even with a $\frac{1}{6}$th objective and $\times 10$ ocular, and very clearly with a 1/12th oil immersion (Winkel-Zeiss).

The mouth aperture is circular and about 2μ in diameter, and leads into an oval buccal capsule about 3μ wide by 5μ in length, which then narrows quite suddenly to form the anterior part of the oesophagus. (Fig. 2, B, O.). These measurements are from contracted specimens examined in 70% alcohol and probably represent the minima.

The buccal stylet is about 7μ long by 1μ broad, and attached to the ventral wall at the base of the buccal capsule; it is divided into two portions, the blade, which is slightly lancet-shaped and flattened dorso-ventrally, but may be twisted in treated specimens; and the shaft, which appears to be slightly broader at the basal attachment (Fig. 2, S).

Fülleborn (1923) states that the shaft in the larval stylet is flattened, but this I have not been able to determine from these adult specimens. The musculature of this region cannot be studied very satisfactorily, but there seems to be no doubt from the examination of living adults that the stylet can be protruded and retracted. Fülleborn (1923) and Li (1923) also supposed that the stylet might be retractile, and in specimens stained in azur-eosin Fülleborn described a violet spot at the junction of the stylet head and shaft, which he suggested might represent the insertion of muscles. The stylet is present in adults of both sexes.

The anterior part of the oesophagus is slender and becomes bent upon itself in a S-shape when the worm contracts, but immediately posterior to the nerve ring the walls become thickened and muscular forming a pseudo-bulb. The lumen of these parts is triradiate, and Chitwood (1931) has described three large nuclei in the walls of the pseudo-bulb, surrounded by granular protoplasm, which appear to be oesophageal gland nuclei. The three muscle sectors of the slender anterior portion are penetrated by three fine ducts, apparently the ducts of these glands in the walls of the pseudo-bulb (Chitwood, 1937).

The pseudo-bulb then narrows very suddenly and bends ventrally at the commencement of the " cell-body " or stichosome, and the oesophagus continues as a fine tube, with muscular walls and discrete nuclei, embedded in the stichosome but not holding a constant position ; it finally emerges at the posterior end of the stichosome to enter the intestine dorsally through the oesophago-intestinal valve. At the junction of the oesophagus and intestine lie the two large cells described by previous writers.

The nature of the stichosome has been determined by Chitwood (1935). Each stichocyte or individual stichosome cell, is a sub-ventral oesophageal gland, which is thus serially reduplicated along the length of the posterior portion of the oesophagus. In Trichocephalus, Chitwood has shown that the protoplasm of each stichocyte is penetrated by fine tubules which join together and open by a single orifice through the wall of the oesophagus into the oesophageal lumen, the orifices of adjacent stichocytes alternating. I have been unable to confirm the presence of these openings into the lumen of the oesophagus in the stichocytes of Trichinella, but in sections of adults fixed in hot Bouin and stained in haematoxylin eosin, distinct tubules and small alveoli may be seen in the stichocytes (Fig. 3, T),

and it seems reasonable to suppose that these join and enter the oesophagus through a single duct as in the related Trichocephalus.

In living adult Trichinella, two types of stichocytes may be distinguished; one type has quite large refringent granules or small globules scattered in the cytoplasm, more numerous towards the centre of the cell (Fig. 1, GS) which stain very deeply with haematoxylin in sections (Fig. 3, SG); while in the other type the cytoplasm is clear or contains only a very few granules. The two types alternate in an irregular manner and probably represent two phases of secretory activity.

Mode of Feeding of the Adults.

Living adults examined in saline at 37°C immediately on removal from the intestine of a freshly killed hamster show clearly the method of feeding, which however, ceases in twenty to thirty minutes after removal.

The anterior slender portion of the oesophagus is extended and shows a very rapid quivering movement which may be seen to be due to quick rhythmic contraction and expansion antero-posteriorly of the buccal capsule region, several times a second. Though the stylet cannot be seen in living specimens, it seems almost certain that this movement of the buccal capsule results in repeated protrusion and retraction of the stylet; this would cause laceration of the tissues round the head of the worm and

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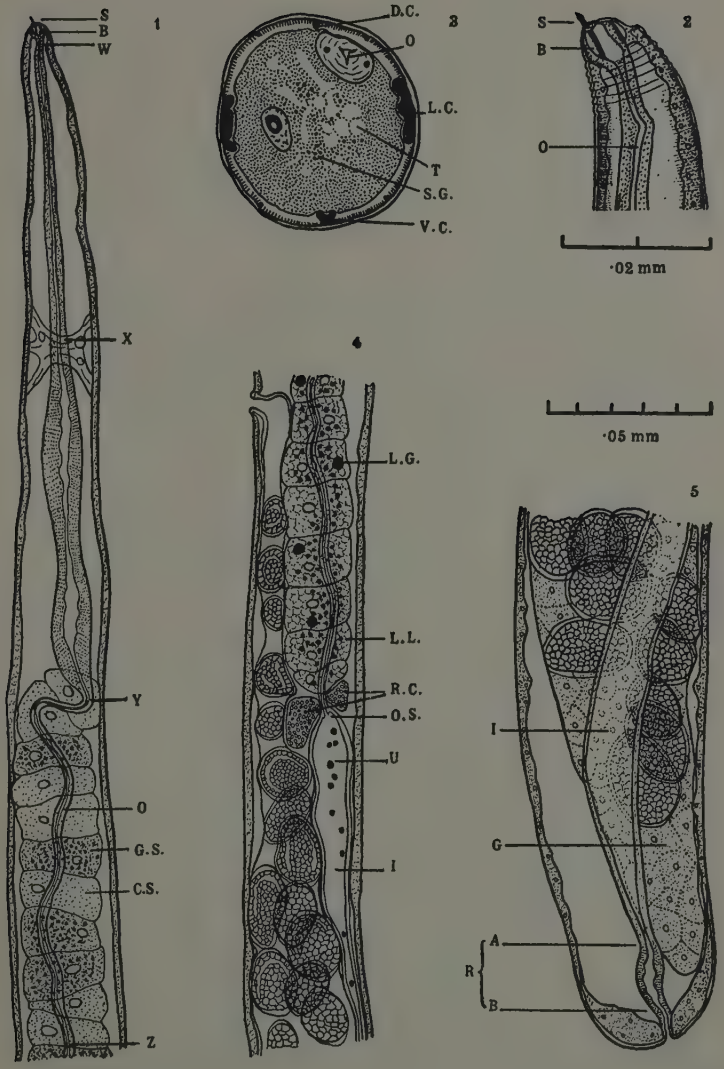
Fig. 1. Anterior region of adult female *Trichinella*. S, stylet; B, buccal capsule region; W–X, slender anterior portion of oesophagus; X–Y, pseudo-bulb of oesophagus, posterior to nerve ring, showing two peristaltic waves of dilatation, one commencing at X and one finishing at Y; Y–Z, slender posterior portion of oesophagus embedded in stichosome, ventral side of worm to right. Anterior third only of stichosome shown. O, oesophagus; GS, granular type stichocyte; CS, non-granular type stichocyte.

Fig. 2.—Anterior end of adult female *Trichinella*, showing buccal capsule and stylet.

Fig. 3.—Transverse section of a stichocyte. DC, dorsal hypodermal chord; LC, lateral hypodermal chord; VC, ventral hypodermal chord; O, oesophagus, with muscular walls and two nuclei (?); T, tubules and alveoli in cytoplasm; SG, granules in cytoplasm.

Fig. 4.—Adult female *Trichinella* showing junction of oesophagus and intestine; LG, large globules in stichocytes; LL, small granules in stichocytes; RC, two large cells at junction of oesophagus and intestine, with granules or globules in cytoplasm; OS, oesophago-intestinal valve; U, globules in intestine; I, intestine.

Fig. 5.—Posterior end of female *Trichinella*; I, intestine; G, gonad; R, rectum, with peristaltic defaecatory movement from A to B.



Morphology of *Trichinella spiralis*.

piercing of small lymphatics and other vessels in the mucosa and submucosa in which the worms live, releasing cell contents and tissue fluids on which the worm probably feeds. This use of the stylet would also enable the parasite to penetrate the mucosa and come to lie buried deep in the submucosa as may be seen in some sections of infected intestine. Such a piercing mechanism would also explain certain features of experiments by McCoy (1936), where larvae injected into the vagina of pregnant rats may be found later in the amniotic fluid of the embryo rats, obviously having penetrated through the amnion.

At the same time the muscular pseudo-bulb shows a rhythmic peristaltic dilatation about once every second from before backwards (in Fig. 1 from X to Y), a movement evidently adapted for the active sucking of fluids through the mouth. This would seem to dispose of Müller's (1929) suggestion that the Trichuroidea feed by osmosis through the anterior part of the body, assisted in some way by the stichosome.

No peristalsis or other movement can be seen in the portion surrounded by stichocytes, or of the oesophago-intestinal valve, or the intestine; the rectum, however (from A to B Fig. 5) shows peristaltic defaecatory movements of its muscular walls, about once every two or three seconds, indicating that either digestion and absorption must be very rapid, or that feeding is very wasteful. A point of some interest is that Giardia individuals, a species of which is common in the hamsters examined, show a tendency to collect round the anus of Trichinella, possibly influenced by the nature of the excretory matter.

The fluid in the oesophagus appears to be brownish pink in colour, but it is difficult to be certain owing to optical effects. It is quite certain, however, that no part of the alimentary canal ever contains solid food particles, except possibly bacteria; and the mouth and oesophagus are obviously too small to accommodate erythrocytes. The food is in a fluid state when ingested, being either tissue fluids, or cell contents, or both, or perhaps predigested tissue acted on by a tissue lysate from the anterior oesophageal glands. These are suppositions only, but Hoeppli (1927, 1933) has suggested that extra-intestinal digestion of host tissue may occur in the case of Trichocephalus, because of the peculiar histological changes seen round the head of the parasite. On the other hand simple laceration of the host tissue by the stylet may account for some of these changes.

Results of Intra-vitam Staining with Neutral Red.

In order to determine something of the function of the stichosome and the reactions of the alimentary canal, some living feeding adults were immersed in dilute neutral red in saline at 37°C. (0·01% solution). Others were placed in dilute Janus green and suspensions of Indian ink, but no conclusive results were obtained by either of these latter methods.

It appears certain that in the adults observed feeding in this medium the neutral red entered by the mouth only, since no other structures except the alimentary canal and related glands were stained in the short time allowed (30 minutes). Had diffusion of the stain taken place through the cuticle or other pores, one would expect staining of the coelomic fluid, hypodermal chords or gonads. These structures were, in fact, stained in worms left in neutral red for an hour or more, indicating slow penetration of some part of the body wall in the living worm. In strong solutions of neutral red no staining occurred at all.

In five minutes the oesophagus is filled to the oesophago-intestinal valve, and the stichocytes commence taking up the stain. It is a curious fact that the posterior stichocytes stain first, and the anterior ones later; the reason for this is obscure, as one would expect them to stain evenly, or even the anterior ones first. Even if the stichocytes performed an absorptive rather than a secretory function this differential staining is unusual; the presence, however, of single small ducts opening into the oesophagus from each stichocyte strongly suggests that their function is secretory rather than absorptive, since the latter function would involve exposure of as large a surface as possible of the cell to the fluid to be absorbed, by analogy with the structure of absorptive surfaces in other animals. A secretory function is also suggested by the histological appearance and position of the granules in the stichocytes.

The granules in the granular stichocytes stain a pinkish red (Fig. 4, LL) and the cytoplasm a diffuse pink, indicating a slightly acid reaction. In worms which have been stained for half-an-hour or more, large globules appear in the stichocytes, which stain an orange-red colour (Fig. 4, LG); these however, appear to be pathological and formed by the confluence of smaller globules, and are not seen in fresh adults.

After fifteen to twenty minutes, small globules, which may also be seen in the unstained living worm, in the intestine and rectum, stain a very deep red colour, showing an acid reaction of these portions of the gut.

The two cells at the junction of oesophagus and intestine are of some interest; Eberth (1860, 1863) described these as unicellular glands with direct openings into the oesophageal lumen, but neither Rauther (1918) nor Chitwood (1937) were able to distinguish any protoplasmic connection of these cells with the oesophageal lining or any tubules in the cytoplasm of these cells, and Chitwood suggests that they may be enlarged mesenterial cells, homologous to the series of smaller cells covering and supporting the oesophagus and stichosome. Results of intra-vitam staining with neutral red, however, lend more support to the suggestion that these are glandular cells which have some functional connection with the oesophagus or intestine.

About fifteen minutes after immersion in the dilute stain, these cells commence to take up the stain, and their cytoplasm appears filled with small globules or granules which stain an orange-yellow (Fig. 4, RC), indicating an alkaline reaction, in marked contrast to the stichocytes and intestinal contents.

The fact that these cells stain simultaneously with the remainder of the alimentary canal and accessory glands, other cells in the body being still unstained, and that they stain in a fashion quite unlike the other cells in the body cavity which stain after prolonged immersion, strongly suggest that these two large cells are functionally connected with the alimentary system, possibly for the secretion of digestive juices.

Reasons have been given on histological and morphological grounds to support the view that the stichocytes are secretory in function, rather than absorptive or stores of reserve food as Chitwood has suggested (1931).

The function of such secretion is uncertain, though the position of the stichocytes *behind* that portion of the oesophagus which acts as a sucking organ would suggest that this secretion is digestive in the oesophagus or intestine, rather than a tissue lysate for extra-intestinal digestion of host tissue. It is probable that the proteins of the plasma, lymph or cell contents on which Trichinella feeds require further simplification before being absorbed in the intestine and assimilated.

The three supposed oesophageal glands in the pseudo-bulb do not appear to stain intra-vitally with neutral red and their function must remain in doubt.

In conclusion my sincere thanks are due to Professor E. Hindle for constant encouragement and advice, and to Professor R. T. Leiper, F.R.S., for helpful suggestions during the course of this study.

Summary.

A functional buccal stylet is described in adult male and female *Trichinella spiralis*, and the mode of feeding of the adults is described from living specimens examined immediately on removal from the intestine. The stylet is rapidly protruded and retracted, probably for lacerating host tissue and releasing tissue fluids, and active peristaltic sucking movements of the pseudo-bulb of the oesophagus take place about once a second. The rectum shows peristaltic defaecatory movements once every two or three seconds. Intra-vitam staining with 0·01% neutral red shows an acid reaction of the stichocytes of the stichosome, which are of a granular or non-granular nature, with small tubules and alveoli in the cytoplasm.

Evidence is adduced on histological and morphological grounds that the stichocytes are secretory rather than absorptive in function, and the two large cells at the junction of oesophagus and intestine which show alkaline cytoplasmic inclusions are probably also functionally connected with the alimentary system as digestive glands. The reaction of the contents of the intestine and rectum is markedly acid.

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Natural Infections of *Heterodera schachtii* on Clovers in Britain.

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In a previous paper (1938) it was recorded that lemon-shaped cysts of the nematode *Heterodera schachtii* are frequently to be found in pasture soils. In many cases the soil has not, at least for a number of years, borne a crop such as oats or mangolds likely to have been infected with this nematode. The eelworm must therefore parasitize some plant or plants commonly to be found in pastures.

In several cases lemon-shaped cysts have now been found attached to the roots of wild white clover, *Trifolium repens* L., and other stages of the parasite have been seen within the tissues of the roots. Natural infections on white, or Dutch, clover were found in two fields at Winches Farm; Dutch clover plants, very kindly brought to the writer by Dr. Goodey from Beckenham in Kent towards the end of October 1938, had white cysts on the roots, and both white and red clover (*T. pratense* L.) growing in soil from Perthshire were found to be infected. It thus appears probable that a strain or strains of *H. schachtii* capable of reproducing on *T. repens* and *T. pratense* occurs widely in Britain. Lemon-shaped cysts of the type which infects clover have been found in soils from Herts, Beds, Kent, Lincs, Yorks, Hants, Ayr, Perth and Jersey, from areas not suspected of being infected with the oat- or beet-strain of *H. schachtii*. The host plants of these cysts are unknown, but red and white clover are so widespread that it is quite possible that, in most cases, they are the hosts.

Morphology of the Clover-Strain Cysts.

The cysts from clover roots, both in the white and brown stages, always have a prominent vulva. In many cases, while the cysts are still attached to the host root, a jelly-like substance can be seen posteriorly, apparently having been exuded from the vulva. This mass is usually about one-quarter to one-half the size of the cyst, and sometimes embryonated eggs are embedded in it. Very often a white " sub-crystalline layer " more or less covers the brown cyst. This can nearly always be

seen when the cysts are still attached to the host root. In colour the cysts are usually dark brown, and in general appearance and size resemble more the oat-strain than other common strains of *H. schachtii*. A minute examination of the cyst walls of ten cysts showed no clearly marked rows of dots, as was the case in cysts from *Agrostis* (Franklin 1938) ; but very fine pit-like markings were faintly visible in some cases. Although most of the cysts are dark brown and have fairly thick walls, some are lighter brown and have thinner walls, through which the eggs can be seen. In some cases, also, the cyst wall is of a much more shiny texture than in others. It is possible that more than one strain of the eelworm has been observed, but amongst cysts which have actually been removed from the roots of *T. repens* there is considerable variation in colour and apparent thickness of the wall.

The length, excluding the neck, of 100 cysts from Hertfordshire soil varied from 0·8 to 0·4 mm., with an average of 0·56 mm. ; from Kent the cysts varied from 0·787 to 0·35 mm. in length, and averaged 0·544 mm. In breadth 100 Hertfordshire cysts varied between 0·525 and 0·25 mm., with an average of 0·384 mm., and the Kentish cysts were from 0·55 to 0·225 mm. broad, with an average of 0·363 mm. In both dimensions the Kentish cysts are thus a little smaller than those from Herts. The ratio of length to breadth varied from 2·125 to 1·122 in 100 Herts cysts, and averaged 1·461 ; the figures for the Kentish cysts were from 1·895 to 1·151, with an average of 1·497. The latter are therefore a little narrower in proportion to their length than the Herts cysts. A comparison of these figures with the corresponding measurements for other lemon-shaped strains of *H. schachtii* shows that the cysts developed on clover are smaller than the other strains. The ratio of length to breadth is rather greater than in pea and oat cysts, but less than in the mangold- and beet-strains : the clover-strain cysts are therefore less spherical than the pea- and oat-strains, but less elongated than the beet- and mangold-strains. The neck is on the average a little longer than that of the pea-strain, and considerably shorter than in other strains, but measurements of the necks of cysts are somewhat arbitrary, as the neck is frequently not straight, and it is difficult to decide on its exact point of origin. The measurements are given in Table I, and drawings of typical clover-strain cysts in fig. 1.

Ten cysts from white clover plants were dissected and the eggs contained in them were counted. The numbers varied from 25 to 403, and

averaged 167·6. One hundred cysts, taken at random from a number which had been removed by flotation from air-dried soil, were dissected and the contents examined. Only five were full of unhatched eggs; 87 were empty, or contained only empty egg shells; of the remaining eight cysts three had approximately three-quarters of the contained eggs unhatched, two had half of the eggs unhatched, and three had a quarter of the eggs unhatched. Thus, in 100 cysts, there was a viability of only 9%.

TABLE I.

Measurements of cysts of some lemon-shaped strains of *Heterodera schachtii*.

Strain.	Average Length in mm.	Average Breadth in mm.	Average Ratio of Length/ Breadth.	Average Length of Neck in mm.
Clover (Kent)	0·544	0·363	1·497	0·061
Clover (Herts)	0·560	0·384	1·461	0·058
Pea	0·605	0·503	1·203	0·051
Oat	0·635	0·446	1·423	0·069
Oat (Triffitt 1929) ...	0·71	0·50	1·4	0·084
Beet (Triffitt 1929) ...	0·73	0·43	1·69	0·86
Mangold (Triffitt 1929) ...	0·84	0·51	1·62	0·077

Eggs and Larvae.

Larvae obtained by crushing the cysts are typical of the species. The average length of 100 larvae, 10 from each of 10 cysts, was 0·491 mm., and the mouth stylet averaged 0·024 mm. in length. The larvae are approximately the size of those of the mangold-strain, but the stylet is a little smaller.

Ten cysts were dissected and from these 100 eggs were measured and averaged 0·098 by 0·040 mm. This is smaller than those of the mangold-strain measured by Triffitt (1929), being nearer the size of the oat-strain recorded by her. (Table II.)

Attempts during August, September and October to hatch larvae from lemon-shaped cysts, obtained from soil, in tap water and in water which had drained through a pot of soil in which white clover was growing were unsuccessful. Clover plants, sown in February in sterilised soil to which had been added lemon-shaped cysts from local soil, were found to

bear cysts when the roots were examined in July. Larvae have, however, hatched within two or three days from cysts removed from clover roots at the end of October and placed at once in a vessel of tap water.

TABLE II.

Measurements of larvae and eggs of different strains of *Heterodera schachtii*.

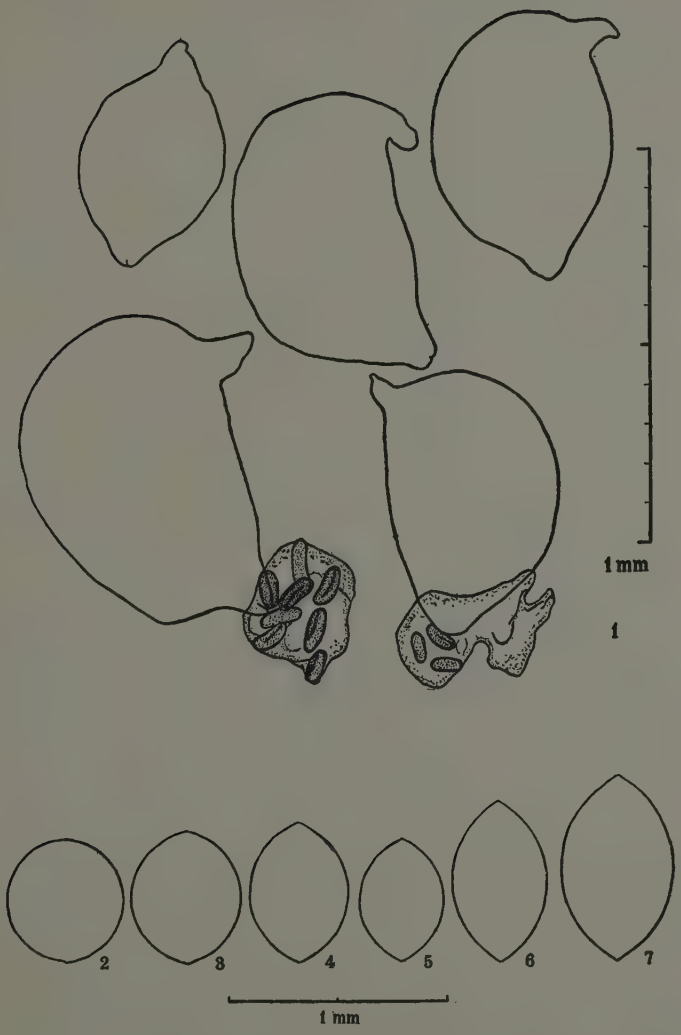
Strain.	Author.	Larvae.		Eggs.	
		Average Length in mm.	Average Length of Stylet in mm.	Average Length in mm.	Average Breadth in mm.
Mangold ...	Triffitt ...	0·50	0·026	0·112	0·046
Clover ...	Franklin ...	0·491	0·024	0·098	0·040
Clover ...	McBeth ...	0·480	—	0·114	0·049
Oat ...	Triffitt ...	0·470	0·022	0·106	0·041
Potato ...	Triffitt ...	0·460	0·023	0·110	0·055
Beet ...	Triffitt ...	0·457	0·024	0·114	0·045

Relationship of the Clover-strain of *H. Schachtii* to other Strains.

While insufficient is as yet known about the cysts of *H. schachtii* which parasitize clover to say whether this is a specialised strain of the eelworm or not, it may be of interest to compare these cysts with those of better known strains. In general appearance the cysts remind one of those of the oat-strain; they are of a similar dark brown colour, and are often encrusted with a white " sub-crystalline layer " such as is also frequently to be observed on newly formed oat-strain cysts. Although they are

Fig. 1.—Cysts from the roots of *Trifolium repens* L. A jelly-like mass with embryonated eggs embedded in it is attached posteriorly to two of the cysts.

Figs. 2–7.—Diagrams representing cysts of six strains of *Heterodera schachtii*. Each is made up from the measurements of the average length and average breadth of the respective strain, the neck being ignored. The posterior protuberance in the lemon-shaped strains is not represented as this is included in the length. (2) Potato-strain. (3) Pea-strain. (4) Oat-strain. (5) Clover-strain (Herts.) (6) Beet-strain. (7) Mangold-strain.



Cysts of various strains of *Heterodera schachtii*.

rather smaller than those oat-strain cysts for which measurements have been recorded, they do not differ greatly from them in the ratio of length/breadth. As compared with the pea-strain, the clover-strain cysts are of a similar length, but rather narrower, and hence the ratio of length/breadth is considerably greater. It is doubtful whether much significance can be given to slight variations in the actual dimensions of eelworms at any stage of their development, as differences almost always occur in the average dimensions even of the same strain parasitizing the same host species. These differences, which are very obvious when one studies the measurements of cysts and larvae of *H. schachtii* given by different authors, may be due to differences in soil conditions, either directly or by their action on the host, or to climatic and other environmental factors. It is very noticeable, when one examines various samples of cysts of the potato-strain, that those from different populations vary in average size. However, they are all more or less spherical. The ratio of length/breadth for a given strain (assuming that the potato-strain is no more than a biological strain of *H. schachtii*) would thus appear to be a more constant feature than the actual size of the cysts. Figs. 2–7 show diagrammatically how the cysts of different strains vary in relative proportions.

Before it was discovered what was the host of the lemon-shaped cysts occurring so frequently in soil, tests were made of various plants which it was thought might be parasitized by them. Negative results were obtained with potato, oats, wheat, sugar beet, various weeds including *Medicago lupulina*, *Rumex acetosa*, *Chenopodium album*, *Lotus corniculatus*, *Polygonum persecaria*, *Plantago lanceolata*, *Ranunculus* sp., Dandelion and about twenty grasses of common occurrence in Britain. In the same soil in which these plants were uninfected *T. repens* became heavily infected in the two or three cases where it was grown, and in the one instance where *T. pratense* was grown this species also was infected. Although a single trial is not conclusive evidence of the immunity of a plant from attack, it seems probable, at least, that the plants mentioned are not readily infected by this strain of *H. schachtii*.

As only two hosts are known for this strain (or strains) of eelworm, it is impossible to say whether or not it could have given rise to any of the strains known to parasitize cultivated plants. It is also impossible to say whether clover-strain cysts might be a source of danger to cultivated crops. Since it appears to occur so widely, it seems probable that if it

readily harmed agricultural crops its presence would have been noticed before now. On the other hand, the occurrence of pea- and oat-strains of *H. schachtii* in many different districts with no indication as to how they got there may be due to natural strains, such as the clover-strain, adapting themselves to the cultivated crop. The widespread occurrence of cysts of *H. schachtii* in soil may, if this is the case, be a real danger to cultivated crops, especially when these are grown year after year on the same land.

Several reports have been published of the occurrence of natural infections of *H. schachtii* in areas where cultivated crops are not known to be attacked. McBeth (1938) has described an infection of undetermined origin on *T. repens* in Utah. He found that the roots which had been heavily infested with eelworms were swollen, and he compared the swellings with the root-galls caused by *H. marioni*. The infected clover plants examined by the writer have shown no more swelling than occurs in the roots of other plants parasitized by *H. schachtii*. Another record of the infection of white clover by *H. schachtii* is that of Hollrung (1890). This worker found a few nematodes on *T. repens* which was grown on land infected with the beet-strain, and concludes that this latter strain is capable of attacking clover. *T. pratense*, *T. hybridum* and *T. incarnatum* were uninfected in his experiments. In the light of the present writer's experience it appears possible that the infection of the clover in Hollrung's experiment may have been a natural one. Other wild plants which have been found bearing *Heterodera* cysts on the roots are *Polygonum* spp., recorded by Steiner (1931), and shadscale, recorded by Thorne (1935) both in the U.S.A.

The presence of naturally occurring strains of *Heterodera* in soils complicates experiments designed to determine the host-range of the various strains and leads one to treat some of the results of such experiments with a certain amount of reserve, except in cases where use is made of sterilised soil infected with cysts of known origin. When soil taken from an " oat-sick " field is used, for example, it may well be that a strain of *Heterodera* parasitizing clover is also present.

With the finding of lemon-shaped eelworm cysts on *T. repens* and *T. pratense* the presence of two distinct strains of *Heterodera* in pasture soils, the one attacking *Agrostis*, the other *Trifolium*, is definitely established. Whether or not they are a source of danger to cultivated plants remains to be seen. Infection experiments with the rounded cysts of the strain

occurring on *Agrostis* (described by the writer in 1938) have given negative results with potato (6 pots), oats, sugar beet, *Mentha arvensis*, *Rumex acetosa*, *Chenopodium album*, *Myosotis* and *Trifolium repens*; also with about 20 grasses including *Agrostis alba* (syn. *A. stolonifera*). The absence of infection on *A. alba* makes it appear that some factor in the experiment may have been unfavourable to the eelworm. The method used has been successful with other strains of *Heterodera*, but it is possible that the cysts were allowed to become too dry after they had been removed from the soil. Potato-strain cysts have been found to be infective after having been kept for several months in a dry state in the laboratory, but the cyst walls of the strain of cysts attacking *Agrostis* appear to be thinner than those of the potato-strain, so it is possible that they are less resistant to drought than the latter. Further tests of the possible host range of these cysts are required.

The clover plant does not appear to be harmed by the parasite, and at present the presence of the lemon-shaped cysts in soils only seems to be of importance as a potential danger to cultivated plants, and as a source of confusion and deception in the examination of soils for the presence of strains of *Heterodera schachtii* harmful to agricultural crops.

The writer wishes to thank Mr. T. Anderson of the Seed Testing and Plant Registration Station, Edinburgh, for kindly sending from Scotland samples of soil containing lemon-shaped cysts. Acknowledgments are also due to the Agricultural Research Council for providing a grant enabling the work to be carried out.

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Experiments on Trap Cropping with Potatoes as a Control measure against Potato Eelworm (*Heterodera schachtii*).

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In a previous paper published in 1937 Carroll & McMahon discussed the possibility of effecting control of potato root eelworm (*Heterodera schachtii*) by means of trap cropping with potatoes. During the past two seasons further experiments, designed with the same object in view, have been carried out. The additional information obtained from these experiments and from the experience of the past two years is presented in the present paper.

In the previous paper it was pointed out that little or no control of the eelworm was effected (and could not be expected) by a method of trap cropping in which potatoes, intended to produce a crop, are planted immediately after a trap crop of potatoes has been removed. Experience during the past two years has conclusively verified this fact and consequently this method of trap cropping does not merit further consideration.

The alternative method of trap cropping is to plant the trap crop of potatoes (or perhaps two successive trap crops) in one year and then to postpone planting a potato crop proper until the next season (or later). Subsequent to the removal of the trap crop some other crop, suitable for late sowing, such as turnips or carrots could be sown.

In the previous paper it was suggested that if trap cropping is contemplated it might be done in such a manner as to necessitate only the removal of the plant with the attached tuber from which it had grown—that it would not be necessary to remove the roots from the soil. The observations which have since been made point definitely to the fact that trap cropping can be successfully carried out without removing the roots, but it has been shown that it is necessary to remove completely the rhizomes from the soil if best results from trap cropping are to be achieved.

If even small pieces of rhizomes are allowed to remain in the soil new growth may start from the buds on these after the removal of the tuber.

In the previous paper it was also suggested that it might be possible to remove the trap crop by just pulling up the potato plant (with attached tuber) by hand. It has since become clear that the plants should be dug out as the rhizomes cannot be removed completely by the pulling out process. It has also been demonstrated that the tubers of the trap crop should be planted fairly near the surface in order that the digging out operation may be more easily and completely accomplished.

It has been shown, as was previously suggested, that trap cropping may be carried out while the soil is devoted to the growing of another crop sown in drills, or widely separated lines. The potato tubers of the trap crop are planted in the furrows, or spaces between the lines, near the surface of the soil, and the young potato plants can be easily dug out at the correct time. It could be arranged to have the first scuffling of the furrows coincide with the digging out of the potato plants. It has not been found practicable to carry out trap cropping during the period while the soil is devoted to the growing of a cereal crop.

From the data which was available at the time of the publication of the previous paper it was stated in that paper " . . . . under natural conditions in the field, plants might be allowed to grow before removal for 30 days after eelworm eggs in the soil commenced to hatch, or about six weeks after tubers are planted (assuming that hatching of larvae commences about 12 days after tubers are planted) without any fear that cysts would be formed from the larvae which hatched during the 30 day period. Possibly no eelworm infection would be left behind in the soil (resulting from development of larvae which hatched) even if the plants were allowed to grow until say about 35 days after hatching commenced."

During the past two years detailed pot experiments have been carried out to ascertain whether the above statement is quite correct.

Length of Time during which the Trap Crop may be allowed to Grow before Removal.

1937 *Experiment.*

In the 1937 experiment sandy soil which was naturally free from eelworm infestation was used. Twelve pots (Nos. 1 to 12 inclusive) were potted up with this soil in the usual manner and a sprouted tuber

was planted in each pot on 3rd May. From 12th May (on which date the sprouts were appearing above the ground), until 29th May larvae, hatching out in the laboratory from about 12,000 cysts (for each pot) were watered into each pot every second or third day for about 18 days.

On 14th June (42 days after planting and 33 days after larvae were first added) the plants and tubers were removed from pots 1, 2 and 3, and the roots remaining in the soil were examined. On 22nd June (50 days after planting and 41 days after larvae were first added) the plants and tubers were removed from pots 4, 5 and 6 and the roots remaining in the soil were examined. On 1st July (59 days after planting and 50 days after larvae were first added) the plants and tubers were removed from pots 7, 8 and 9 and the roots remaining in the soil were examined. The plants in pots 10, 11 and 12 were allowed to grow on to the end of the season.

After the first examination of the roots, made on the day when the plants were removed from the pots, the roots were in each case put back into the pots from which they came and a re-examination of the roots of all the plants was made on 14th July.

The following observations were made :—

Pots 1, 2 *and* 3.

Examination on 14*th June*.—No cysts could be seen on the roots and no mature females could be seen breaking through the surface of the roots.

Examination on 14*th July*.—A fair number of very small yellow and brown cysts were seen on the roots. This examination demonstrated that some of the eelworms in the roots continued to develop and eventually produce very small cysts after the roots had been severed from the growing plant.

Pots 4, 5 *and* 6.

Examination on 22*nd June*.—An abundance of small cysts (mostly white and some turning yellow) were found on the roots.

Examination on 14*th July*.—An abundance of yellow and brown cysts were found on the roots. These cysts were smaller in size than those in the succeeding pots.

Pots 7, 8 *and* 9.

Examination on 1*st July*.—An abundance of good sized yellow cysts were found on the roots.

Examination on 14th July.—An abundance of normal sized yellow and brown cysts were found on the roots.

After the examination on 14th July the roots were again in each case put back into the pots from which they had come. During the following winter, the soil (and root remains) in each pot was thoroughly mixed and rubbed down. The contents of each three similar pots was then thoroughly mixed ; three soil samples were taken from each lot and cyst counts were made.

The following is a summary of the cyst counts :—

Soil from pots 1, 2 *and* 3.—2·2 very small cysts per c.c. of soil with an average content of about 25 eggs. This would represent a residual infection of about 55 *eggs per c.c. of soil.*

Soil from pots 4, 5 *and* 6.—4·2 cysts per c.c. of soil with an average content of about 80 eggs. This would represent a residual infection of about 340 *eggs per c.c. of soil.*

Soil from pots 7, 8 *and* 9.—6·5 cysts per c.c. of soil with an average content of about 140 eggs. This would represent a residual infection of about 900 *eggs per c.c. of soil.*

Soil from pots 10, 11 *and* 12.—5·8 cysts per c.c. of soil with an average content of about 140 eggs. This would represent a residual infection of about 800 *eggs per c.c. of soil.*

The above figures are self-explanatory and summarise the results of the experiment. Two points of outstanding interest are to be noted, viz. :—

(*a*) A slight soil infection results if the plants are allowed to grow, before removal, for 33 days after the roots have first been invaded by larvae.

(*b*) The residual soil infection in pots 10, 11 and 12 (from which the plants were not removed) was not greater than the residual infection in pots 7, 8 and 9 (from which the plants were removed 50 days after larvae had first invaded the roots).

1938 *Experiments.*

In 1938 four distinct experiments were carried out, viz. :—

(A) Using soil naturally free from eelworm, planting in April, and adding larvae.

(B) Using soil naturally infected with eelworm and planting in April.

(C) Using soil naturally free from eelworm, planting in May, and adding larvae.

(D) Using soil naturally infected with eelworm and planting in May.

For experiment A, six pots (Nos. 70 to 75) were potted up in the usual manner and a sprouted tuber was planted in each on 7th April. From 18th April larvae, hatching out in the laboratory from about 12,000 cysts (for each pot), were watered into each pot every second or third day for 10 days.

On 14th May (26 days after adding the first larvae) the plant in pot No. 70 was rooted out and its roots were carefully examined under a binocular microscope. No mature female eelworms could be found.

On 17th May (29 days after adding the first larvae) the roots of the plant in pot 71 were similarly examined. No mature females could be found.

On 20th May (32 days after adding the first larvae) the roots of the plant in pot 72 were examined. On this date some mature females were found just breaking through the surface of the roots.

For experiment B, ten pots (Nos. 76 to 85) were potted up and a sprouted tuber was planted in each on 7th April. Commencing on 5th May (with pot 76) one pot was turned out every second day and the roots of the plant carefully examined under a binocular microscope. No mature female eelworms were found until 20th May (bursting through the surface of roots in pot 83) *i.e.*, until 43 days after planting.

For experiment C, six pots (Nos. 86 to 91) were potted up and a sprouted tuber was planted in each on 10th May. From 23rd May larvae, hatching out in the laboratory from about 12,000 cysts (for each pot), were watered into each pot every second or third day for 10 days.

On 14th June (22 days after adding the first larvae) the roots of the plant in pot 86 were carefully examined under a binocular microscope. No mature female eelworms were found.

On 16th June (24 days after adding the first larvae) the roots of the plant in pot 87 were similarly examined. On this date mature females were found just breaking through the surface of the roots. These roots after they had been detached from the plant were put aside in a Petri dish with a little water and kept under observation for some weeks. It was found that the females which were first visible on 16th June eventually developed into very small cysts having an average content of under 30 eggs.

For experiment D, ten pots (Nos. 92 to 101) were potted up and a sprouted tuber was planted in each on 10th May. Root examinations

were commenced on 5th June, the intention being to examine the roots of one plant every second day. On 9th June (30 days after planting) mature female eelworms were found bursting through the surface of the roots.

These 1938 pot experiments demonstrate the very much more rapid development of *Heterodera schachtii* in the warmer month of May than in the month of April. In naturally infected soil the period elapsing between the date of April planting (7th April) and the finding of the first mature females was 43 days, whereas female development was completed in 30 days after the May planting (10th May). This is corroborated by the experiments in which larvae were watered into soil naturally free from eelworm infection. Development of the females in the roots took 32 days in the case where larvae were first added to the soil on 18th April, but was completed in 24 days in the case where larvae were first added to the soil on 23rd May.

By comparing the 1937 and 1938 pot experiments it will be seen that the length of time required for completion of development of *Heterodera schachtii* was longer in 1937. In that year the first larvae were added to the soil (naturally free from infection) on 12th May, and 33 days later (on 14th June) mature females could not be seen. In 1938 the first larvae were added on 23rd May and in this case mature females were seen breaking through the surface of the roots 24 days later. The fact that the first larvae were added 11 days later in 1938 could hardly by itself explain the quicker development. It is most likely that the 1938 season was more favourable for rapid development of the eelworms.

In the previous paper by the present authors it was stated :—" From the experiment it is reasonable to expect that the degree of eelworm infestation in the soil could be enormously reduced by putting in a trap crop of potatoes and removing the young plants and tubers (from which they grew) about six weeks after the tubers were planted." In the light of the 1937 and 1938 experiments and from experience gained since the publication of the previous paper it is clear that the above statement cannot be allowed to stand as a recommendation.

It is felt now, however, that the following definite recommendations can be made as regards the removal of a trap crop, assuming in every case that nicely sprouted tubers have been planted :—

(*a*) If the tubers of the trap crop are planted in April the trap crop should be removed from the soil *five weeks* after planting.

(*b*) If the tubers of the trap crop are not planted until after 1st May the trap crop should be removed from the soil *four weeks* after planting.

(*c*) If it is sought to produce in one season the greatest possible effect from trap cropping by planting a second trap crop immediately after the removal of the first, the procedure should be as follows :—plant the tubers of the first trap crop early in April and remove that crop *five weeks* later. Plant the second trap crop immediately after the removal of the first and remove the second trap crop *three weeks* after planting. It is necessary to remove the second trap crop three weeks after planting on account of the fact that eelworm larvae are actually hatching at the time when the second trap crop is being planted. Consequently the roots can be invaded immediately and development completed in a little over three weeks.

The above recommendations have been made as a result of experiments carried out in the Dublin area. Possibly they may have to be modified slightly for other areas owing to variation in climatic conditions.

Preliminary Observations on the Effect of Trap Cropping.

Pot Experiments.—Reference to the previous paper will reveal that in 1936 the following pot experiments were laid down using for the purpose naturally infected eelworm soil as it came from the field :—

In 4 pots (Nos. 51 to 54) tubers were planted on 7th April, and plants were removed on 20th May (43 days later).

In 4 pots (Nos. 55 to 58) tubers were planted on 7th April, and plants were removed on 3rd June (57 days later).

In 4 pots (Nos. 59 to 62) tubers were planted on 7th April, and plants were removed on 12th June (66 days later).

There were also control pots which were allowed to grow on to the end of the season.

In 1937 all these pots were replanted with potatoes and as the season progressed notes were made regarding the growth and appearance of the plants.

The plants in the control pots and also those in pots 59, 60, 61, 62 developed typical bad symptoms of eelworm attack showing that no obvious improvement was produced by removing the trap crop plants 66 days after an April planting.

The plants in pots 55, 56, 57, 58 showed typical symptoms of eelworm attack but they were not so bad as 59, 60, 61, 62, indicating that some diminution in the intensity of the attack, but not obvious control, was produced by removing the trap crop plants 57 days after an April planting.

The plants in pots 51, 52, 53, 54 were much better than the others in general growth and appearance and remained in an active growing condition for a much longer period, indicating that a very obvious degree of control was obtained by removing the trap crop plants 43 days after an April planting.

Unfortunately during a period towards the end of the season when the pots were not being regularly observed they were attacked by rats and consequently it is not possible to give the yield of tubers produced (later it will be mentioned that rats also attacked the experimental plots).

In 1937 six pots (Nos. 13 to 18 inclusive) were potted up with naturally infected eelworm soil and a sprouted tuber was planted in each on 3rd May. Pots No. 13 and No. 14 were allowed to grow on to the end of the season as controls. On 14th June (42 days after planting) the plants and tubers were removed from pots 15, 16, 17 and 18 and a second lot of sprouted tubers was replanted in these pots on the same day. On 13th July (29 days after second planting) the plants and tubers of the second trap crop were removed. The pots, including control pots, were then put aside for replanting in 1938.

From the data obtained from other 1937 pot experiments it was realised at the end of the 1937 season that a control of potato eelworm could not be expected in pots 15, 16, 17 and 18 following the removal of two trap crop plants from each pot. Reference to these other pot experiments, carried out in 1937 and already described in this paper, where planting of the tubers was also done on 3rd May will make it clear why a control could not be expected. It will be seen that following a May planting the first trap crop plants should have been removed much sooner than 42 days after planting and it is now clear also that the second trap crop plants should not have been allowed more than three weeks growth. However, the pots 13 to 18 were replanted in 1938 and the result was as expected. The plants in all the pots showed typical severe symptoms of potato sickness and it was considered that no purpose would be served by weighing the tubers produced in each pot.

Plot Experiments.—All the plots mentioned in these experiments were artificially made and were of the same size, viz., 8 ft. × 4 ft. Each plot was prepared by excavating an area 8 ft. × 4 ft. to a depth of about 15 ins. The pit so excavated was then lined around the sides with boards and afterwards filled with naturally infected eelworm soil of a sandy nature brought in from a field where severe symptoms of potato sickness had manifested themselves. Sufficient soil to fill all the pits was first collected and mixed thoroughly by many turnings so that at the commencement of the experiments the soil in each plot was quite uniform as regards cyst content. Throughout all the experiments 27 tubers, evenly spaced out, were planted in each plot and the manurial treatment of each plot remained constant throughout.

In the spring of 1936 five plots were laid down as follows:—

Plot 1. A crop of carrots was grown in 1936.

Plot 2. Potatoes were planted on 23rd April and allowed to grow to end of season.

Plot 3. Potatoes were planted as a trap crop on 23rd April. This trap crop was removed on 3rd June and a second crop of potatoes (which was allowed to grow to end of season) was planted immediately afterwards.

Plot 4. Potatoes were planted as a trap crop on 23rd April. This trap crop was removed on 3rd June and carrots were afterwards sown in the plot.

In both plots 2 and 3 the potatoes which were allowed to grow to the end of the season exhibited obvious symptoms of potato sickness. The weight of tubers produced in plot 2 was 22 lb. and the weight produced in plot 3 was 14 lb. This demonstrates a point already emphasized, namely, that no advantage whatever results from planting a crop of potatoes immediately after the removal of a trap crop. The yields from these two plots may seem unusually high for potatoes growing in eelworm infected soil, but can probably be accounted for by the fact that the plots were heavily manured with farm yard manure and well sprouted tubers were planted.

In 1937, on 10th May, each of the four plots of the previous year were planted with potatoes and the crop in each plot was allowed to grow on to the end of the season. In addition to these four plots another plot No. 5) was laid down in 1937 with naturally infected eelworm soil.

In plot 5 potatoes were planted as a trap crop on 10th May. This trap crop was removed on 22nd June (42 days after planting), but on this date there were white cysts visible on the roots. A second trap crop was planted immediately after the removal of the first and this second trap crop was removed on 14th July (22 days after planting). After the removal of the second trap crop the plot was allowed to remain fallow for the remainder of the season. As regards appearance of overground growth the potatoes growing in plots 1, 2, 3, and 4 did not differ very markedly from one another, but plot 4 would rank as best. It was hoped that the yield of tubers obtained from each plot would supply additional useful information, but unfortunately the plots were raided by rats towards the end of the growing season and consequently the yields remain an unknown quantity.

For the 1938 plot experiments it was realised that no purpose would be served by continuing plots 1, 2, 3 and 4. Accordingly the soil was removed from each of them and the soil from the four plots was thoroughly mixed together by being turned over several times. The mixed uniform soil was then replaced in plot 1, 2, 3 and 4. Plot 5 which had carried two trap crops in 1937 was allowed to remain untouched.

As an addition to the experiment five new plots (Nos. 6 to 10 inclusive) were laid down in 1938 with thoroughly mixed, naturally infected, eelworm soil brought in from the field.

The scheme for the 1938 plot experiments was as follows :—

Plot 1. Control plot. Potatoes were planted on 8th April and allowed to grow to the end of the season.

Plot 2. Potatoes were planted as a trap crop on 8th April and also beet seed was sown between the rows of potato tubers. On 13th May the potato plants were removed.

Plot 3. Potatoes were planted as a trap crop on 8th April and the plants were removed on 13th May. Turnip seed was sown after the removal of the potato plants.

Plot 4. Two trap crops of potatoes were taken from this plot. The tubers of the first trap crop were planted on 8th April and the plants were removed on 13th May. The tubers of the second trap crop were planted on 14th May and the plants were removed on 10th June. Turnips were sown after the removal of the second trap crop.

Plot 5. Plot which had been trapped cropped twice in 1937. Potatoes were planted on 8th April and allowed to grow to the end of the season.

Plot 6. Control plot same as plot 1.

Plot 7. Same as plot 2.

Plot 8. Same as plot 3.

Plot 9. Same as plot 4.

Plot 10. Potato tubers were planted in this plot as a trap crop on 8th April and on the same date the plot was seeded with oats. On 13th May the potato plants were removed.

Discussion on the 1938 Plot Experiments.

In only 3 plots, viz., Nos. 1, 5 and 6 were the potatoes allowed to grow to the end of the season. The potatoes in these plots were sprayed as a preventive against potato blight. The plots were dug out and the produce weighed on 6th September.

From the experience with plots 2 and 7 it was apparent that it would be quite feasible in practice to remove a trap crop of potatoes from between the lines of such another crop as beet, turnips, etc.

From the experience with plot 10 it was apparent that it would not be practicable to remove satisfactorily a trap crop of potatoes from a cereal crop.

Plots 2, 3, 4, 7, 8, 9 and 10 which were trap cropped with potatoes in 1938 will be planted with potatoes in 1939.

Plot 1. The potatoes in this plot exhibited very uneven growth throughout the season. Most of the plants had obvious symptoms of potato sickness, but some were not very obviously diseased. Yield of tubers obtained was 11 lb.

Plot 6. The growth of the potatoes in this plot was poor and symptoms of potato sickness were obvious. Yield of tubers obtained was 8 lb.

Plot 5. Two trap crops of potatoes had been removed in 1937. The potatoes growing in this plot in 1938 were quite normal and were very healthy looking. Yield of tubers obtained was 20 lb.

The marked superiority of plot 5 was not fully expected because, as is mentioned earlier in this paper, white cysts were already formed on the roots of the first trap crop in 1937 before the plants of that trap crop were removed. It can be stated however that in removing these plants the great bulk of the roots was also removed, and as the white

cysts were at the time very firmly attached to the roots very few of them would have been left behind in the soil. Consequently almost the full effects of a properly executed double trap cropping would have been secured.

It is realised that the data so far obtained from plot experiments on trap cropping is very limited, but nevertheless it is felt that the results are encouraging. Experiments have proved that an April planted trap crop should be removed five weeks after planting and consequently the time during which a trap crop occupies the ground (even when planted by itself) is not unduly long. It should be a point of considerable interest that two successive trap crops can be removed in one year in so short a period as eight weeks from the date of planting of the first trap crop.

In conclusion the authors desire to call particular attention to the points enumerated at the beginning of this paper particularly the necessity for removing completely the rhizomes when digging out the plants of the trap crop.

Reference.

Carroll, J. & McMahon, E., 1937.—" Potato Eelworm : (*Heterodera schachtii*) Further Investigations." *J. Helminth.*, xv (1) 21–34. (*W.L.* 112246.)

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The Treatment of Seed Potatoes for the Destruction of adherent *Heterodera schachtii* Cysts.

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The importance of arresting the spread of the potato-strain of *Heterodera schachtii*, which is largely responsible for the serious disease known as "potato sickness," has frequently been emphasised. The cysts of this nematode, as is well known, can withstand long periods of drought, and can therefore be carried about in a viable condition on any object to which small lumps of soil may become attached. In this way the eelworms may be spread, not only from one field to the next by means of farm implements, but also from one locality to another many miles away in soil adhering to seed potato tubers. Seed potatoes grown on eelworm-infected land, however free from disease in other respects, are therefore highly undesirable for planting on clean land, unless some treatment can be carried out which will kill adhering cysts. It was with the object of discovering some practical means of treating seed potatoes so that, without injury to the tubers, adhering eelworm cysts would be killed, that the experiments recorded here were undertaken.

The problem was approached simultaneously along three lines, namely, the effects of the various treatments on cysts *in vitro*, on clean seed potatoes and on cysts adhering in soil to potato tubers. Obviously, a process which will kill eelworm cysts on potatoes but will also damage the tubers would be useless.

Three types of treatment were adopted—hot water, cold chemical solutions, and hot chemical solutions. Only such treatments were used as were thought would prove fairly harmless to the potato.

Treatment of Cysts *in vitro*.

In each test 100 cysts were used. The cysts were used dry, as they would be in this state when carried on seed potato tubers. After treatment they were put in dishes of "potato root excretion," that is to say dishes containing water which had percolated through soil in which a potato was growing. At frequent intervals the dishes were examined for hatched

larvae. Fresh potato root excretion was substituted as necessary and the examinations were continued for several months, except in cases where the treatment had obviously been ineffective.

1. *Hot water.*

The thermal death point of the larvae within the cysts of *H. schachtii* was worked out by Triffitt & Hurst (1935). They found that in order to kill the cyst contents it was necessary to immerse the cysts in water at 116°F. for at least 45 minutes : at 118°F. and 120°F. 30 minutes were necessary, and at 130°F. 5 minutes was the lethal period. Hot water treatments at least as long as these would therefore be necessary to kill cysts carried on potato tubers.

2. *Phenol.*

Cysts immersed in a 5% phenol solution for 1 and 2 hours failed to produce any larvae during 7½ months in potato root excretion which was renewed at intervals. This treatment was therefore lethal to the cyst contents.

3. *Mercuric chloride.*

Exposure to a 0·2% solution of mercuric chloride for 2 hours failed to kill the entire contents of cysts ; hatching was delayed for about 10 weeks, but over 550 larvae hatched during the following 9 weeks. The effects of longer exposure to this chemical were not ascertained.

4. *Iodine.*

Cysts were soaked in a 5% solution of N/10 iodine in potassium iodide for periods up to 6 hours. This treatment apparently had no adverse effect on the cyst contents as hatching took place normally.

5. *Formalin.*

Numerous trials of formalin solutions of various strengths have been carried out. Commercial formaldehyde (40%) was used and diluted as required.

Eelworm cysts were first exposed in a closed vessel to the vapour given off from formaldehyde for periods up to 4 hours. Cysts thus treated were not killed, although larvae did not begin to hatch from those exposed for the longest period until over 4 weeks had elapsed from immersion in the first dose of potato root excretion. Cysts were next exposed for 8 hours to a 0·5% formalin solution, but this did not kill all the larvae, although hatching was again delayed for a month. Seven

hours in a 1% formalin solution also failed to kill the cyst contents, though no hatching occurred for 7 weeks, and then only 79 larvae appeared during 14 months. A 2% solution of formalin did not entirely kill the contents of cysts which had soaked in it for 4 hours, though only 26 larvae hatched from the 100 cysts during 14 months, the first of these appearing after 10 weeks' immersion in potato root excretion. Five hours in 2% formalin was, however, lethal to the cyst contents. After 2 hours in 5% formalin larvae hatched from cysts after 6 weeks had elapsed, but 3 hours and over in the solution appeared to kill all the larvae. Soaking in 10% formalin for 2 hours and in 20% or 40% formalin for 1 hour was lethal to the cyst contents, but is likely to be too drastic for application to seed potato tubers.

6. *Hot Formalin Solutions.*

It was thought that the addition of formalin to a hot water bath might reduce the length of time or the temperature necessary for treating potatoes for the killing of cysts. It was found that immersion in a 0·5% solution of formalin for 30 minutes at 116°F., or for 15 minutes at 118°F. or 120°F. was necessary to kill the contents of cysts. Ten minutes at 118°F. or 120°F. was insufficient, though 7 weeks elapsed before hatching began. When a 1% formalin solution was used at 124°F., 5 minutes were required to kill the cysts, and at a temperature between 134° and 139°F. about 2½ minutes exposure was lethal. The period of time necessary for killing the cyst contents in the hot solution of formalin was thus rather less than when water only was used, and very much less than with cold formalin solutions.

It is interesting to note that a delay of over 7 weeks before hatching started was observed in the case of cysts which had been treated in 0·5% formalin for 10 minutes at 118°F.; several hundred larvae hatched in subsequent weeks: ten weeks delay occurred after treatment with 0·2% mercuric chloride for two hours: Smedley (1939) also records delays of six weeks and two months before hatching began from cysts treated with isothiocyanates. These considerable delays which sometimes occur before hatching takes place show the necessity for continuing the stimulation of cysts for at least three months after any treatment in order to be certain of its effect.

The treatments which were found to be successful in killing cysts *in vitro* are summarised in Table I.

TABLE I.

Treatments lethal to cysts of *Heterodera schachtii*.

Treatment.	Solution.	Temperature.	Duration of Treatment.
Hot water (Triffitt & Hurst)	Water	116°F.	45 minutes.
,, ,, ,, ,,	,,	118°F.	30 ,,
,, ,, ,, ,,	,,	120°F.	30 ,,
,, ,, ,, ,,	,,	130°F.	5 ,,
Formalin	2%	Room	5 hours.
,, ...	5%	,,	3 ,,
,, ...	10%	,,	2 ,,
,, ...	20%	,,	1 ,,
,, ...	40%	,,	1 ,,
Phenol	5%	,,	1 ,,
Hot formalin	0·5%	116°F.	30 minutes.
,, ,, ...	0·5%	118°F.	15 ,,
,, ,, ...	0·5%	120°F.	15 ,,
,, ,, ...	1%	124°F.	5 ,,
,, ,, ...	1%	134°–9°F.	2½ ,,

Treatment of Potato Tubers.

In the application of hot water or chemical treatments to seed potatoes two factors have to be studied—the time of year when the treatment is applied, and the variety of potato used. Some varieties may withstand high temperatures and chemicals better than others. It is of course desirable that the treatment should be suitable for application to any variety of potato without risk of damage.

The period of possible application of the treatment extends from the time when the tubers are lifted to planting time. It is probable that the most convenient time for treatment will be when the seed is spread out for sprouting, or immediately before planting. If the earlier time is chosen, the tubers should be allowed to drain before being stored in the boxes, so that fungi may not be encouraged to develop.

Clean seed tubers were used and planted in soil free from *H. schachtii*.

1. *Hot Water.*

The hot water bath employed was of the bulb-bath type with a removable wire basket for the potatoes. The water was kept hot by means of a thermostatically controlled gas burner. The temperature of the water was raised above that at which it was required to treat the tubers so that when the potatoes were put in it went down to the required level, where it could be kept for the necessary length of time. For the

killing of eelworm cysts it is of course only important that the outside of the tuber should be heated.

Two of the hot water treatments which had been shown to be effective in killing cysts were applied to seed tubers of seven varieties in March 1936. The temperatures used were 118°F. for 30 minutes, and 130°F. for 8 minutes, and the varieties were Eclipse, Sharpe's Express, Arran Comrade, British Queen, Majestic, King Edward and Golden Wonder. There were thus included in the trial both early and maincrop varieties. Twenty tubers of each variety were used for each treatment. After treatment the tubers were spread out in boxes to sprout for 2 or 3 weeks ; they were then planted in a field plot, the varieties being kept separate. There were 3 rows of each variety, one of each of the two treatments and a control row. When the crop was lifted at the beginning of October the yield from each plant was weighed separately.

Taking all the varieties together 91·8% of the control tubers grew, 85·7% of those treated at 130°F. for 8 minutes and 72·1% of those treated at 118°F. for 30 minutes. The average yield per plant for all the varieties together was 13·3 ozs. for the control, 11 ozs. for those treated at 118°F. for 30 minutes, and 9·6 ozs. for those treated 130°F. for 8 minutes. There was thus a reduction of 17·3% and 28% respectively in the yields from potatoes subjected to the two treatments. Of the seven varieties used King Edward suffered most as a result of the treatments ; Majestic was rather less adversely affected ; Eclipse, Arran Comrade and British Queen were only slightly affected. The fact that the treatments were given rather late in the season might account for the adverse effect on the yields and the failure of some of the tubers to grow.

The following year experiments were carried out earlier with small numbers of six of the varieties which had previously been used, Golden Wonder being omitted. This time three tubers of each variety were heated for 15 minutes in water at 130°F. and a similar series for 10 minutes in water at 135°F. The treatments were carried out on January 18th, and repeated on February 13th and March 15th. The tubers were spread out to sprout after treatment and were examined at intervals to see how they were progressing. Some damage was evident in all cases, and the shoots, which had begun to grow before the March treatments, were killed at the tips, but in many cases lateral buds developed in their place. Larger numbers of thinner shoots were produced which would of course not be so strong as the original ones. The results of these experiments, as shown

by the condition of the tubers at planting time, indicate that the treatments had a rather adverse effect on the potatoes, especially when applied in February and March, and in particular the higher temperature was harmful. King Edward was again the most severely affected variety. As the number of tubers of each variety and in each trial was small, no record was made of the yields.

A further trial of the effect of hot water on the growth of seed potatoes was made in November 1937. This time only the variety Majestic was used, and 10 tubers were used for each treatment. The tubers were immersed in the water at temperatures of 120°F., 130°F. and 135°F. for 30, 20 and 15 minutes respectively. Those treated at 135°F. for 15 minutes were all killed: at 130°F. for 20 minutes 3 of the ten treated survived, but these were late in sprouting and weak: at 120°F. for 30 minutes only one was killed, the other 9 being fairly vigorous. These 9 tubers gave an average yield of 3·07 ozs. per plant as compared with an average of 4·64 ozs. per plant from 10 control tubers. All the plants were grown in plant pots. Ten Majestic tubers immersed in hot water at 125°–126°F. for 20 minutes on January 14th were delayed in growth and produced weak shoots, but only one failed altogether. The average yield of these plants per tuber, grown in pots, was 4·17 ozs., as compared with the control of 4·64 ozs.—a reduction of about 10%.

Thus, even when treated in November, tubers subjected to temperatures of 130°F. or over for 15 to 20 minutes or more were somewhat damaged. Since 5 minutes exposure at 130°F is necessary to kill cysts when they are separated from soil and immersed alone in hot water, a longer period would be required if the cysts were embedded in soil adhering to a potato which would at first be cold and would take a little while to become warm. This period would probably be not less than 10 minutes, which is dangerously near the time which is harmful to the potato. Lower temperatures would therefore be preferable if hot water treatments are to be used for potatoes. At 120°F. 30 minutes exposure is necessary to kill cysts *in vitro*, but this treatment, though not lethal to the seed tubers, when applied in November, resulted in an appreciable reduction in the yield from plants grown in plant pots. The reduction in yield when tubers were treated in January at 125°–6°F. for 20 minutes was less marked, but there was a definite delay in sprouting. It is of course recognised that growth in plant pots which are not more than 7 inches in upper diameter, as were the ones used in these experiments,

is quite inadequate for potato plants, but it is probable that the yield gives some idea of the vigour of the plants as compared with that of the controls.

It thus appears that at the temperatures and for the times required to kill eelworm cysts carried on potato tubers by immersion in hot water, the tubers are adversely affected. Experiments on the hot water treatment of potatoes, published in the Annual Reports of the Seale-Hayne Agricultural College for the years 1933, '35, '36 and '37 indicate that immersion for 1 hour at 110°F. in February or March results in a reduced yield, but an increased yield was obtained when the treatment was carried out for 20 minutes at the end of January. It is evident, however, from the work of Triffitt & Hurst that exposure to such a temperature for 1 hour is insufficient to kill eelworm cysts, or even to retard hatching of the larvae. It seems probable that much longer than 1 hour at this temperature would be necessary to kill the cysts, and it is doubtful whether the potato tubers would stand such treatment, at least if treated in the early part of the year. It is possible that if the treatment were carried out before January it would be less harmful.

2. *Phenol.*

Steeping in 5% phenol solution for 3 hours in November followed by rinsing in running water proved lethal to Majestic tubers.

3. *Formalin.*

The first trials of the effects of immersing potato tubers in solutions of formalin at room temperatures were carried out in April. Tubers treated with 0·5% formalin for 8 hours survived, but others treated with 1% and stronger solutions failed to grow. The potatoes were of the variety Great Scot. In January of the following year, 1937, three tubers of each of the six varieties previously used were immersed in 1% formalin for 8 hours and equal numbers in 2% formalin for 5 hours. Similar treatments were carried out in February and March of the same year. Those treated in January suffered no apparent damage, and at planting time appeared to be as well advanced as the controls. Similarly, those treated in March did not seem to have been adversely affected. Some of the tubers treated in February, however, were slightly damaged : Express, Majestic and King Edward were rather delayed in sprouting, and one or two Majestic and King Edward tubers failed to grow. Express tubers had the original shoots killed at the tips, but laterals had grown

at the time when the tubers were planted. The other three varieties, Eclipse, Arran Comrade and British Queen, seemed unharmed.

During the winter of 1937-8 Majestic tubers were treated with a 5% solution of formalin : 10 tubers were immersed for 5 hours in November and 10 others for 6 hours in January. All the tubers grew well and were planted in pots. The average yield per pot for the 20 plants was rather less than that of the controls—3·67 ozs. as compared with 4·64 ozs.

A larger-scale treatment of Majestic tubers was carried out in February. About 120 tubers were treated for 6 hours in 5% formalin, and after being kept in sprouting boxes for some weeks were planted out in field plots in April. At the time of planting 75% of the tubers were well sprouted and appeared as good as the controls. Of the remaining thirty tubers ten grew later. It is probable, judging by the small-scale experiments, that a smaller proportion of the tubers would have failed if the treatment had been carried out in January or earlier. The tubers were planted in 7 plots of 12 tubers each, the plots being arranged at random mixed with plots of control tubers. The average yield from the treated tubers was 22 lbs. 1 oz. per plot, and from the ten control plots 18 lbs. 13 ozs. The yields from the individual plots were :—

Control tubers. (Plots of 12 plants)		Treated tubers. (Plots of 12 plants)	
lbs.	ozs.	lbs.	ozs.
17	0	21	8
13	8	23	8
17	0	25	0
21	0	23	8
16	8	21	8
19	4	11	8
28	0	28	0
21	0		
21	0		
13	4		

4. *Hot Formalin.*

As exposure of eelworm cysts to certain hot dilute solutions of formalin seemed to be effective in killing the contents, experiments were made to determine whether potato tubers would withstand this treatment. In January 1937 three potatoes each of the six varieties used before were immersed in 0·5% formalin at a temperature of 130·5°F. for 5 minutes,

and a second series was treated in a 1% solution at 130°F. for 5 minutes; similar treatments were repeated in the middle of February and the middle of March. In all cases the tubers survived treatment, but those treated in February and March in most cases had the original shoots more or less damaged, though lateral buds developed in their place. The January treatments were not appreciably different in appearance from the controls.

TABLE II.

Treatments apparently harmless to potato tubers.

Variety of potato.	Date of treatment.	Solution.	Temperature.	Duration of treatment.
Six varieties	Jan. 18th and Mar. 15th.	1% formalin	Room	8 hours.
,, ,,	Jan. 18th and Mar. 15th.	2% ,,	,,	5 ,,
Majestic ...	Nov. 2nd ...	5% ,,	,,	5 ,,
,, ...	Jan. 11th ...	5% ,,	,,	6 ,,
Six varieties	Jan. 18th ...	0·5% ,,	130·5°F.	5 minutes.
,, ,,	Jan. 18th ...	1% ,,	130°F.	5 ,,
Majestic ...	Jan. 11th ...	1% ,,	120°–121°F.	20 ,,

In the following season further tests were carried out with Majestic tubers. In November 10 tubers each were exposed to 1% formalin at 120°F. for 20 minutes, 130°F. for 15 minutes and 135°F. for 10 minutes; in January a similar number was treated for 20 minutes in 1% formalin at 120°–121°F. Of those treated at 135°F. for 10 minutes only 6 grew. These did not grow very strongly, and, having been grown in plant pots, gave a yield considerably below that of the controls. Those treated at 130°F. for 15 minutes gave a rather better yield, but again only 6 out of 10 survived. Only 1 tuber treated for 20 minutes at 120°F. in 1% formalin failed to grow. Those which grew yielded an average of 4·10 ozs. per tuber in plant pots, as compared with 4·64 ozs. for the controls. The later treatment at 120°–121°F. for 20 minutes gave a yield of 4·52 ozs. and all 10 plants grew. A larger-scale treatment on February 18th of 120 tubers in 1% formalin at 120°F. for 20 minutes was less successful. When they should have been ready for planting out in the field plots in the third week of April, only 10 tubers had sprouted well. Two months later 53 had sprouted, but the shoots were nearly all very weak. It is possible that, with the larger number of tubers treated, the temperature in the hot water bath was not uniform, and may have risen above 120°F.

in parts and have damaged the tubers in those places. Whether this was so or not it seems apparent that it is important for this type of treatment to be carried out early in the season, probably not later than January. Treatment in November also seems to be preferable to February or March treatment.

Table II summarises the treatments which were apparently harmless to the tubers.

Treatment of Potato Tubers Bearing Cysts.

In certain of the foregoing experiments *H. schachtii* cysts were attached to the potato tubers before treatment; the tubers were subsequently grown in plant pots, and, when the tops were beginning to die down, the roots were examined for the presence of eelworm cysts. In this way the effectiveness of the treatments in killing cysts adhering to seed tubers could be judged.

The cysts were removed by the usual flotation process from air-dried soil and divided into lots of about 100–200. Each lot of cysts was then mixed with a small quantity of dried powdered soil—about as much as would go on a farthing—and the soil and cyst mixture was moistened and smeared on to the tuber to be treated. A piece of butter muslin about 1 inch wide was then tied over the patch of soil and knotted on the other side of the potato, in order to prevent the cysts from being washed off during treatment. In this way the potato probably had a much larger number of cysts, much more securely attached and better protected than it could naturally carry. When the tubers were ready for planting, the " bandages " were removed and any loose cysts were planted with the tuber in a pot of sterilised soil. The soil had been sterilised several weeks before.

In the first series of experiments done in this way a large proportion of plants grown from tubers which had been subjected to treatments which had been shown to kill cysts *in vitro* were infected. For example, seven of 17 tubers which had been immersed in water at 130°F. for 15 minutes bore cysts on the roots, though the average number was only 15 from an infection of 100–200. Eighteen other tubers soaked in 1% formalin for 8¾ hours were all infected, having on the average 41 cysts per plant; 9 of 18 tubers treated with 2% formalin for 5 hours had an average of 26 cysts per plant. The probable explanation of this failure was that the

soil used in attaching the cysts to the tubers was applied too thickly; in some cases it was as much as $\frac{1}{8}$ inch thick. In later experiments a thinner layer of soil has been used, more nearly approaching the amount which would normally be present on a seed tuber. In the second series of experiments new cysts removed from the roots of the previous year's potato crop were used and attached to the tubers to be treated as described above. After $13\frac{1}{2}$ weeks' growth the plants were beginning to die down and the roots were examined for cysts. Control plants grown from untreated tubers to which cysts had been artificially attached had a good infection on the roots. Plants grown from tubers which had been subjected to the following treatments were free from infection:—5% formalin at room temperature for 6 hours and for 5 hours; hot water at 130°F. for 20 minutes, 125°F. for 20 minutes and 120°F. for 30 minutes; 1% formalin at 135°F. for 10 minutes, 130°F. for 15 minutes and 120°F. for 20 minutes. In Table III these results are set out, together with the numbers of tubers which survived each treatment and the dates of treatment.

TABLE III.

Treatments successful in killing cysts embedded in soil on Majestic tubers.

Treatment.	Temperature.	Duration of treatment.	Number of tubers surviving out of ten treated.	Date of treatment.
Hot water ...	130°F.	20 minutes	3	Nov. 3rd.
,, ,, ...	125°F.	20 ,,	9	Jan. 14th.
,, ,, ...	120°F.	30 ,,	9	Nov. 3rd.
Formalin 5% ...	Room	5 hours	10	Nov. 2nd.
,, 5% ...	,,	6 ,,	10	Jan. 11th.
,, 1% ...	135°F.	10 minutes	6	Nov. 3rd.
,, 1% ...	130°F.	15 ,,	6	Nov. 3rd.
,, 1% ...	120°F.	20 ,,	7	Nov. 3rd.
,, 1% ...	120°F.	20 ,,	10	Jan. 14th.

Discussion.

By comparing the treatments apparently harmless to potato tubers given in Table II with those lethal to cysts adhering to tubers, given in Table III, it can be seen what methods are likely to be of use in rendering seed tubers grown in eelworm infected soil fit to be planted in clean land.

Hot water treatments at the temperatures tried and applied in November or January are ruled out as being harmful to the tubers. It appears very improbable that any hot water treatment that is harmless to the potato will be sufficiently powerful to kill adherent eelworm cysts.

The two remaining effective methods of treatment, namely, immersion in hot or cold formalin solutions seem to offer more promise of success. Soaking in a solution of 1% commercial formaldehyde at 125°F. for 20 minutes in January, or in a 5% solution at room temperature for 5 or 6 hours in November or January, seems to have been harmless to Majestic tubers, yet effective in killing attached cysts.

It is obvious that to soak large quantities of seed potatoes in cold formalin solution is a very much simpler process to carry out than to subject them to a hot formalin treatment. Although the cold treatment should take 6 hours and would require more formaldehyde than the hot method, it would need no expensive equipment and no skilled manipulation. The potatoes could be treated before sprouting has begun, so that the sprouts would not be damaged during treatment. One necessary precaution is that large lumps of soil should be removed. It is unusual for such lumps to be present on seed tubers, but if the tubers are rather dirty some means must be taken to get rid of as much soil as possible before treatment. Washing in running water or possibly riddling dry to shake off the lumps of soil, which generally loosen on drying, would be suitable methods. Cysts embedded in small quantities of soil will be killed by the hot or cold formalin treatments. It should be pointed out that in only one experiment in which tubers were successfully treated with cold formalin were the plants subsequently grown in the field and the yield weighed. In this case the plots of treated tubers gave an average yield over 17% greater than that from the control plots. The fact that, in the small-scale experiments which were considered to be harmless, the plants grown from treated tubers were generally as healthy in appearance as the controls, and that the development of the shoots during the sprouting period was not delayed, indicates that a satisfactory yield would probably have been obtained had the plants been grown in the field. The numbers of plants in the pot experiments were too few for much significance to be attached to the yields recorded. It is very improbable that when tubers show no signs of damage before planting the yield will be adversely affected.

In order to arrest the spread of eelworm to uninfected land, all seed potatoes grown in localities in which there is the slightest suspicion of the presence of any strain of *Heterodera schachtii* harmful to cultivated crops should be treated in some way to kill the cysts which are bound to be carried on them. Steeping in cold 5% formalin solution for 5 or 6 hours appears at present to be the most suitable method if carried out before the beginning of February. This would also be a convenient way of treating sacks and implements suspected of carrying eelworm cysts, though in the case of implements large clods of soil should of course be detached. When the soil has become thoroughly saturated with the formalin solution 5 hours soaking will kill any cysts present in it.

Another use to which the formalin bath could be put with advantage is for the steeping of infected potato roots when the crop is being dug. If the roots are collected before they become dry, and put at once into a bath of 5% formalin for 5 to 6 hours a very large number of eelworm cysts will be killed. The roots must however be collected immediately after they have been uncovered, as it is well known that the mature cysts become detached very easily once the roots have dried. For this reason, immediate immersion in formalin would be an infinitely more useful practice than that sometimes carried out of burning the roots a day or two after they have been dug. In infected potato-growing areas it would be well worth the trouble and expense to do this, as a large proportion of the newly formed cysts would be destroyed instead of adding to the infection in the soil.

The writer is indebted to Professor R. T. Leiper, F.R.S., for suggesting the combined use of heat and chemical treatment and to the Agricultural Research Council, for a grant which made it possible for this work to be carried out.

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On the Structure of the Cyst Wall of *Heterodera schachtii* (Schmidt).

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The examination under the high power of the microscope of the walls of *Heterodera* cysts found on the roots of *Agrostis stolonifera* L. (Franklin 1938) showed the presence of rows of markings having the appearance of small punctations, and led to the tentative diagnosis of these cysts as *H. punctata* Thorne. By the kindness of Dr. Thorne the writer has been able to compare her cysts with some of Dr. Thorne's material, and has found that the surface markings have the same appearance. The writer would like here to acknowledge her indebtedness to Dr. Thorne for kindly lending his material. Cysts of several strains of *H. schachtii* have since been examined to find out if they also bear distinctive markings of any kind.

Surface View of Cyst Wall.—The method of examination was to break open the cysts, arrange pieces of the wall on a slide in glycerine and examine them with an oil immersion lens having a magnification of about 1,100 diameters.

It was soon evident that cysts of the potato strain of *H. schachtii* which are in good condition are plainly marked with dots arranged in rows (fig. 2). These are much the same in appearance as those on cysts of *H. punctata* (fig. 1), and *H. schachtii* cysts from tomato roots have similar markings. On the inner surface of the cyst wall the dotted markings cannot be seen, and the wall usually has a rather indefinite appearance, probably due to the remains of the internal structures of the worm.

None of the other strains of eelworm examined show regularly arranged dots, but all have similar, but often finer, pit-like markings distributed evenly over the surface of the wall (figs. 3 & 4). Cysts from the following

hosts were examined : oat, pea, sugar beet, brassica, myosotis, carnation, marram grass and lemon-shaped cysts from pasture soil, probably parasites of wild clover. No differences could be detected between the markings on cysts of these strains.

In order to find out if the pit-like markings are merely due to an optical effect, pieces of cyst wall were examined in liquids with refractive indices of from 1·3 to 1·75. The markings, however, remained visible in all the liquids. Cysts were then treated with sodium hypochlorite solution. This chemical will dissolve the cyst wall (Smedley 1936) and the idea was to dissolve the outer layer of the wall and to examine the deeper layers which would be left. Cysts were floated in a solution containing about 1% available chlorine and kept under observation. After approximately 20 minutes the part of the cyst which had been submerged had become bleached and the outer layers of the wall had been dissolved, while the upper part which had remained above the solution was of course unaffected and could be compared with the treated area. The cyst was broken open, the eggs removed and the wall flattened out on a slide and examined. The pit-like marks were visible on the part which had not come into contact with the chemical, but none could be seen where chemical action had taken place. The inside of the wall of a cyst was then treated with sodium hypochlorite while the outside was left exposed to the air. This was done by floating small pieces of the cyst, inside downwards, on drops of the chemical under the microscope. After a short time the wall became thin, transparent and almost colourless. On examination the pit-like marks were still visible all over the outside of the wall. At the edges of pieces of cysts treated in this way areas could be found where the thin remaining part of the wall was actually perforated (fig. 5). This appearance was obtained with potato strain cysts and also with the pea and oat strains. From these observations it seems probable that the dotted markings are pits or dents of a shallow nature on the outer surface of the cyst wall.

Another character frequently to be seen in cysts of all strains when examined at a high magnification is what appears to be a series of ridges on the surface of the wall (fig. 6). These form a rather irregular network on the main body of the cyst, while in the vulva and neck regions they appear to be arranged more or less in parallel wavy lines, running round the cyst. They are sometimes quite faint, but in most cysts they are

well defined. It is thought that their clearness may be influenced by the degree of desiccation of the cyst, or possibly by its age. Newly formed cysts usually show these markings clearly, but in old ones they may be very faint. These reticulate marks have been seen in cysts of all strains of *Heterodera* which have been examined, including *H. punctata.*

Transverse Sections of Cyst Walls.—Transverse sections of cyst walls of potato strain cysts show two distinct layers in the wall. The outer broader layer is a darker brown than the inner and appears to have faint radial striations. It is possible that these may be the pits observed in surface views of the cyst, but the sections were not thin enough to establish this point. The inner region tends to split into three or four layers running round the cyst and on the inner side it has a rather uneven outline. In favourable sections the reticulate appearance observed in surface views of the cyst wall can be seen to be due to shallow ridges or corrugations on the outside of the cyst. The way in which these ridges are arranged at the anterior and posterior ends of the cyst, where least swelling has taken place, and their relative distance apart in these positions, together give the impression that they may have developed from the larval striations. When the female swells the striations are likely to be much distorted and the original arrangement may be destroyed.

Pea and oat strain cysts were examined in transverse section and it was found that in these too the outer wall of the cyst is covered with a network of ridges which were, however, more pronounced in the sections observed than in the potato strain cysts. Two layers in the cyst wall can usually be distinguished, but the inner is often very narrow. The outer appears to be radially striated and occasionally in these sections as well as in sections of potato strain cysts, narrow wavy lines, having the appearance of very fine channels, can be seen traversing the wall. The exact nature of these lines was not determined.

The Sub-crystalline Layer.—Many observers have mentioned the white flaky layer which is often to be found coating newly formed mature cysts of most strains of *H. schachtii.* It occurs on the beet, oat and pea strains, and has been seen on cysts from marram grass and white clover; it is also present on *H. punctata.* This coating is, however, absent or present only in the very early stages on cysts developing on potatoes and tomatoes.

area of a slightly darker tone than the rest of the cyst, in the middle of which is a small opening. Over 200 potato strain cysts have been dissected for the purpose of observing this feature, and except in the case of a few old decayed cysts it has always been possible to find the aperture. If the portion of cyst wall including the dark region is separated and flattened on a slide, the single hole can be clearly seen ; in diameter it is in the region of 17μ. If the material is examined under the 1/6th objective of a compound microscope, a second minute aperture is sometimes seen beside the large one at a distance of about the diameter of the latter from it. A short groove on the inside of the cyst wall can sometimes be seen leading towards this from the anterior end of the cyst. This small aperture may be the remains of the anus, while the larger has been the vulva, though to be certain of this point young females should be examined.

The vulva can also be found quite easily in the rounded *Heterodera* cysts from pasture soil, but instead of there being one large and one minute aperture there are two holes of approximately equal size lying close together. Both are easily seen at a magnification of 32 diameters, and were observed in all but 3 or 4 of over 100 cysts which were dissected. Presumably, in this race the anus is much bigger than it is in the potato strain, or is much less reduced during cyst formation.

In all cysts it is usual to find round the vulval and anal apertures knob-like projections on the inside of the cyst wall. These appear to be of the same material as the wall and help to give the dark appearance surrounding the apertures. It is thought that they may originally have been points of attachment for muscles.

An examination of the necks of cysts shows that a small aperture remains at the anterior end in the position of the mouth opening.

It may be of interest to note here that larvae have often been observed emerging from potato strain cysts and that usually they come out through the posterior aperture, though they have also been seen making their exit through the neck of the cyst. These two places would presumably be the easiest means of exit and would also be the points at which water and root excretions would be most likely to enter and stimulate the larvae to hatch from the eggs.

Discussion.

At the outset it was hoped that some characteristic features of the cyst wall might be found which would serve to distinguish cysts of the various strains of *H. schachtii* from one another. Goffart (1934) describes how the oat and beet strains may be distinguished by differences in colour and dimensions of the cysts, and by the shape of the eggs and graphed measurements of the larvae. A fairly large number of cysts would be required to determine the strain by this method, and it was thought that if there were any constant differences in the structure or markings of the cyst wall these would serve as a quicker means of determination, and could be used to identify empty or nearly empty cysts.

All the strains examined fall into two groups: in the one there are pits arranged in rows running round the cysts equatorially, while in the other the pits are irregularly placed, and tend to be somewhat smaller. In the former group is *H. punctata* together with the rounded potato and tomato strain of *H. schachtii*; in the latter are the lemon-shaped strains of *H. schachtii* parasitising oats, peas, sugar beet, brassica, carnation, myosotis, marram grass and clover. Since, however, these two groups of cysts can be distinguished from one another macroscopically by their shape, the difference in surface markings of the walls is unimportant for this purpose. Within the two groups of cysts the various lemon-shaped strains are difficult to distinguish from one another by any one definite character, but in the rounded types of cyst the presence of one posterior aperture in the potato strain and two in cysts parasitic on grass—features easy to observe at a fairly low magnification—forms a convenient distinction.

It remains to be decided whether the potato strain of *Heterodera schachtii* should be included in the same species as the lemon-shaped forms attacking oats, peas, beet, etc., or whether it should be considered as a separate species. If the latter, then should it be grouped in the same species as *H. punctata*? It resembles the latter in the lack of a prominent vulva and in the possession of regular rows of pit-like markings on the cyst wall, but differs in having only one posterior aperture as against two in *H. punctata*, and also in having the larval tail less pointed than in the latter (Franklin 1938). Whether *H. punctata* from Canada also differs from *H. schachtii* in these two respects or not is at present unknown, therefore no definite decision can be arrived at.

These preliminary investigations, while demonstrating the presence of pitting on the surface of eelworm cysts, do not explain how the pitting arises. Since the investigations of Chatin in 1887 and 1888, and of Fuchs in 1911, as far as is known, the development of the brown cyst from the white stage female in *H. schachtii* has not been followed. Fuchs states that in the beet strain refractive droplets are exuded through pores in the epidermis of the female, and that they coalesce and congeal to form a covering over the dead worm. It is hoped that it may be possible to follow the development of the brown cyst of the potato strain of *H. schachtii* to find out if a similar process occurs, and whether there is any significance attached to the presence of the rows of pits, and also to determine the exact origin of the posterior apertures. It is possible that a more detailed knowledge of the development of the brown cyst may reveal differences between the so-called strains of *H. schachtii* which, added to those already known, may be sufficiently important to necessitate the distinguishing of two or more separate species.

This work has been carried out under the direction of Professor Leiper, F.R.S., and with the aid of a grant from the Agricultural Research Council. The photographs were taken by Mr. W. M. McDonald, to whom the writer is very grateful.

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What is *Cephalobus parasiticus* Sandground 1939 ?

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Introduction.

It is doubtless a mere truism to state that the helminthologist may experience considerable difficulty in the accurate identification of the worms encountered in the course of his work. Particularly is there the risk of confusion in the case of nematodes occurring in the alimentary canal of animals, for here there is a chance that they may be free-living or saprophagous forms which have been taken in with the animal's food. For the recognition and determination of such nematodes, therefore, a wide acquaintance with various groups of nematodes, including saprophagous forms, is very desirable.

In line with the foregoing, is the view expressed in the following extract taken from the opening paragraph of a recent paper by Sandground (1939). " Experience has shown that if the pitfalls of experimental work with some of the parasitic nematodes are to be avoided, helminthologists should possess a sufficient knowledge of the morphological and biological characteristics of the free living nematodes inhabiting water, soil and decomposing organic material as well as nematodes, parasites or semiparasites of plants."

These words are so germane to the subject of that paper as to justify their quotation by way of preface to some considerations upon it in the following communication.

Sandground found in the stomachs of 11 out of 16 monkeys (*Macaca irus mordax* syn. *M. cynomolgus*) caught in the vicinity of Batavia, Java, an abundance of nematodes, including adult males and females and larvae, of which he gives an excellent illustrated description and which he places in the genus *Cephalobus* under the name of *Cephalobus parasiticus* n. sp. They occurred also in fewer numbers throughout the intestine from the duodenum to the colon. They were apparently not pathogenic to the host since the mucosa of the stomach wall was found to be uninjured

and intact. A test animal was successfully infected by administering adult worms and larvae to it through a gastric sound but an attempt to infect 4 young albino rats and a guinea pig was unsuccessful. It was found that the nematodes could be cultured fairly easily in Petri dishes on a bouillon-agar medium at room temperature, i.e. at about 26° to 31°C. Sandground concludes that the worms may be regarded as saprozoic and facultatively parasitic in monkeys. Elsewhere in the paper he says :—" Despite the implications of the specific name it is conceivable that this species may also be found leading a non-parasitic existence."

The purpose of the present paper is to suggest that the worms do not belong to the genus *Cephalobus* but to the genus *Turbatrix* established by Peters (1927) for the vinegar eelworm and other species previously placed in the genus *Anguillula*; that they are, in fact, most probably the sour paste worm, *Turbatrix rediviva* (Linn., 1767) Peters, 1927 syn. *Anguillula rediviva* (Linn., 1767) Stiles & Hassall, 1905 of which the writer published a description several years ago (see Goodey, 1922).

Morphology.

The following are the chief features in which *Cephalobus parasiticus* is found to agree with *Turbatrix rediviva*.

1. *Shape.*—Adults of both sexes have the same general shape; the body tapering a little anteriorly and considerably posteriorly to the narrow pointed tail.

2. *Size.*

C. parasiticus		*T. rediviva*	
Length	Female 900–1450μ	Length	Female 1040–1370μ
	Male 780–1120μ		Male 950–1240μ
Width	Female 35–48μ	Width	Female 52–60μ
	Male 33–40μ		Male 38–52μ

According to these measurements, *T. rediviva* appears to be a little stouter than *C. parasiticus* but the worms under cultural conditions show a good range in width and older females are often much wider than young ones.

3. *Cuticle and Striations.*—Sandground says that in *C. parasiticus* " the cuticle appears to be devoid of striae but is really very delicately striated as can be seen under highest magnifications in favourable preparations." The writer found the cuticle to be very finely striated, the striae being visible under an oil-immersion lens.

4. *Head.*—In *C. parasiticus* "the mouth is surrounded by 6 inconspicuous rounded lips each carrying a barely perceptible cephalic papilla." In *T. rediviva* the mouth is surrounded by 6 rounded lips. In his 1922 paper the writer stated that there are no oral papillae and it would seem to be highly probable that these small papillae were overlooked.

5. *Buccal Cavity.*—In *C. parasiticus* this consists of a "relatively cylindrical chamber 7μ by about 5μ, with thin filamentous walls and conspicuous though short refractile cuticular rods (telorhabdions) at the base where the vestibule narrows to form an infundibular part." In *T. rediviva* the writer figured a similar simple buccal cavity the walls of which appear in optical section to bear slight lateral thickenings practically at the base of the cavity where it joins the oesophagus.

6. *Oesophagus.*—Sandground's fig. 1 of *C. parasiticus* shows an oseophagus having the same shape and structure as that possessed by *T. rediviva* except that the isthmus is a little longer than that shown in the writer's figure of the organ (l.c. 1922, fig. 1). In both there is an anterior fusiform, muscular corpus in the walls of which there are supporting strands or sheets which are bifid anteriorly. The isthmus is of variable length, however, and in many specimens is longer than the region represented in that figure.

It is to be admitted that, at first sight, the buccal and oeseophageal characters of *T. rediviva* are not unlike those of certain cephalobs particularly of *Panagrolaimus rigidus* syn. *Cephalobus rigidus* (see Thorne, 1937).

7. *Female Characters.*—Sandground's fig. 2 of an entire female of *C. parasiticus* shows that the vulva is situated at 2/3 of the body length from the anterior end. In this it agrees with the position of the vuvla in *T. rediviva.* The muscular vagina leads inwards and forwards and at its junction with the uterus gives off, on its dorsal side, a posterior, blind uterine sac which stretches almost half way to the end of the intestine. Exactly the same arrangement of structures was described by the writer for *T. rediviva.* Anteriorly the gonad consists of a tubular uterus in which occur, according to the degree of development reached by the worm, a variable number of ova; those nearest the vagina often being fully embryonated or larvae may lie free in the uterus. In front the uterus is expanded into a receptaculum seminis and the ovary is then reflexed backwards in the body; the end of the ovarian rachis often extending

beyond the level of the anus. The receptaculum seminis and the prolonged backward extension of the ovary were not described in the writer's 1922 paper.

8. *Male Characters.*—It was Sandground's drawing of the male tail of *C. parasiticus* (his fig. 3) and of an additional spicule which first attracted the writer's attention. The spicules are knobbed anteriorly; the knob having a kind of ventral hook. The posterior end of each spicule is bifid and the concave ventral side of each carries a thin transparent membrane. The description and figures of these structures agree very well with those given by the writer (l.c.) for the spicules of *T. rediviva*. In the matter of size also they agree fairly well with those of *T. rediviva*. Sandground gives, length of spicules, 65–70 μ and gubernaculum, 25–28μ. There appears, however, to be some considerable variation in the length of the spicules. That represented in the writer's fig. 3 A (1922 paper) has a length of about 47μ whilst that shown in his fig. 4 A has a length of about 70μ which agrees closely with Sandground's measurement. A gubernaculum length of 25–28μ given by the writer is the same as that given by Sandground for this structure.

The disposition of the caudal papillae on the male tail is also practically the same in *C. parasiticus* and *T. rediviva*. Sandground figures 6 pairs of papillae for *C. parasiticus* whereas the writer figured only 5 pairs for *T. rediviva*. Of these 1 and 2 are sub-ventral and pre-anal, 3 and 4 are sub-ventral and post-anal, whilst 5 is sub-dorsal and post-anal. The extra pair figured by Sandground is sub-ventral and immediately post-anal.

The general conclusion drawn by the writer from a consideration of the foregoing points is that, apart from the somewhat variable size of the spicules and the absence of the 6th pair of caudal papillae from *T. rediviva*, the agreement in anatomical features between *C. parasiticus* and *T. rediviva* is so close as to suggest their probable identity. In order to clarify the position, therefore, the writer formally proposes *C. parasiticus* as a synonym of *T. rediviva*.

Biological Considerations.

At first sight it may seem remarkable and not a little strange that the sour paste eelworm should be found alive in the stomachs of wild monkeys in a country, moreover, from which this species, so far as the writer is aware, has not previously been reported. Facts, however, which have come to the writer's knowledge in recent months concerning the occurrence

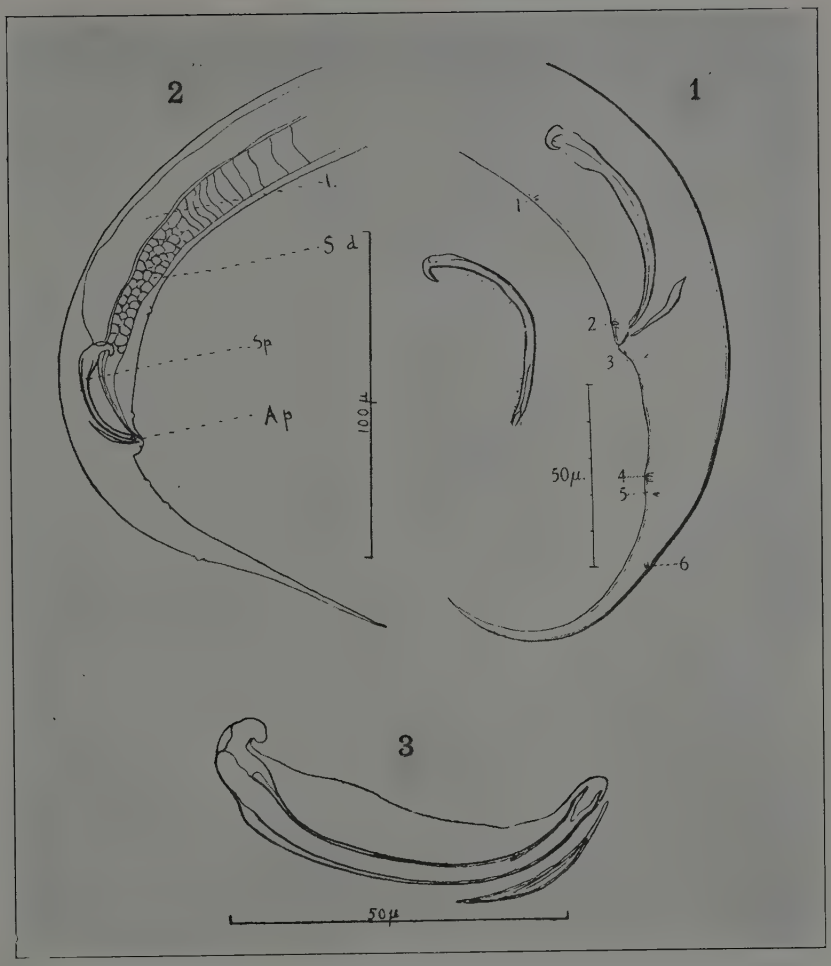

Fig. 1. *Cephalobus parasiticus* Sandground, 1939. Male tail and an additional spicule, in lateral aspect. After Sandground. (This fig. is reproduced from *Parasitology* by permission of the Cambridge University Press.)

Figs. 2 and 3.—*Turbatrix rediviva* (Linn. 1767) Peters, 1927. Fig. 2. Male tail in lateral aspect. Fig. 3. A single spicule and gubernaculum more highly magnified. Both after Goodey, 1922.

To face page 138.

of this, or a very closely related nematode, may help to elucidate the matter and even render plausible a suggestion as to how the monkeys may have acquired the nematodes in their wild state.

It is first necessary to point out that the so-called sour paste worm is not confined to paperhanger's paste or similar substances though it has, up to the present, been described only from such media which must become infected with the nematodes on being exposed to the open air. That the worms do occur under natural conditions is proved by the following facts though the actual substances on which they occur in the wild state are at present unknown. Between 1922 and the autumn of 1938 the writer had not seen living specimens of the paste eelworm but in October, 1938, material was sent from a correspondent at Cambridge University, with a request for the identification of the nematodes which were present in large numbers in certain cultures used for breeding Drosophila flies. One of these cultures consisted originally of some crushed banana and a little maize meal which had been moistened with cyder ; the other only of crushed banana moistened with cyder. Both cultures had been exposed in the laboratory in order to attract Drosophila flies from the outside and when the flies had laid their eggs in the dishes containing the cultures the latter were bottled. After about 3 weeks the nematodes were first noticed in the cultures. It is feasible to suggest that, originally, they had been introduced into the cultures by the visiting flies which had somehow become contaminated with them in the wild state. It is at this point that our knowledge of the occurrence of the worms in nature breaks down but it is reasonable to suppose that there must be substances, such as fruits or other bodies rich in sugar, which in undergoing an acid fermentation, would afford a suitable habitat for them and also for Drosophila, and that the latter, in flying about in search of fresh breeding places, actually carry the nematodes with them.

Confirmation of this suggestion is to be found in a paper by Aubertot (1925) on the closely related nematode of beer filters, *Turbatrix silusiae* (de Man), which he found in the vicinity of Strasbourg, in acid cultures of a purée of potato, exposed with a view to attracting Drosophila flies. These cultures became contaminated with *T. silusiae* brought to them on the bodies of the visiting flies and Aubertot found that he could grow the worms equally well on flour paste, on banana or on potato purée. He also states that although the worms cannot resist desiccation yet he

found living larvae in old dried cultures and he goes on to suggest that, in all probability, some of the eggs within a female worm may remain alive under conditions of desiccation on a fly's body and that it is from these that fresh infections may be set up. It seems to be fairly well established that Drosophila flies can carry nematodes on their bodies in a viable condition and so may take them to new sources of food where they can live and multiply.

Now Macaque monkeys are vegetarian mammals and we may presume that in the wild state they eat fruit and vegetables of various kinds. Assuming such food to be partially decayed or undergoing an acid fermentation and to be infected with *T. rediviva* brought to it, in the manner suggested above, by fruit flies, it is easy to see how the monkeys caught in the environs of Batavia may have acquired the eelworms of this species found in their stomachs. In the light of this last suggestion, Sandground's words, quoted in the introduction to the present paper, that " it is conceivable that this species may also be found leading a non-parasitic existence " are perfectly relevant. One may venture to suggest, further, that banana or other suitable substances exposed at Batavia with a view to attracting fruit flies might prove a fruitful source of investigation from which one might be led eventually to the discovery of the natural habitat of the worms in question. The fruits usually eaten by these monkeys might also repay close investigation.

In order to render these biological considerations complete, mention must be made of another species of *Turbatrix* already known to occur in the Dutch East Indies namely, *Turbatrix nepenthicola* (Menzel) syn. *Anguillula nepenthicola* Menzel, which has been found in the hanging pitchers of the pitcher-plant, *Nepenthes gymnamphora* Nees. An account of this species is to be found in a paper by Micoletzky & Menzel (1928) in which Micoletzky gives a technical description of the worms whilst Menzel contributes an account of their biology. The worms differ from *T. rediviva* in certain well defined features. The adults are larger than those of *T. rediviva* ; the female being from 2·3 to 2·88 mm. and the male 1·36 to 1·87 mm. long ; the spicules, though of the same general shape and with bifid tips, have knobbed heads without a ventral hook and the male tail carries 7 pairs of papillae. Of these, 1, 2 and 3 are sub-ventral and pre-anal, 4, 5 and 6 are sub-ventral and post-anal, forming a compact group not far from the tip of the tail, whilst 7 is a smaller papilla and is lateral and post-anal.

The species appears to be most nearly related to the beer filter nematode, *T. silusiae*, from which, however, it differs in the shape of the spicules and in the distribution of the papillae of the male tail.

Biologically the worms are interesting as Menzel mentions that they have been found only in pitchers hanging from trees and situated from 3 to 6 metres above ground, being absent from those forming rosettes at ground level. In the pitchers containing them there also occurred numerous wings of dipterous insects and it would seem quite possible, or even probable, that in this case also the nematodes may have been conveyed to the pitchers by visiting flies. Had the worms found in the stomachs of the monkeys turned out to belong to this species rather than to *T. rediviva* a new and extremely interesting sidelight might have been thrown on the habits of the monkeys in question as we should have had to visualize them taking liquid refreshment in the forest from the hanging pitchers of pitcher-plants ; a feat probably not outside the realm of possibility but one the necessity for which we must, unfortunately, abandon.

As to the ability of *T. rediviva* to live at the temperature of the monkey's body, it must be admitted that no exact information is at present available. Sandground mentions that the worms found by him though relatively easy of culture on a bouillon-agar at 26°–31°C. did not fare so well on cultures maintained in the 37°C. incubator which he attributed to an over abundant growth of certain bacteria inimical to the nematodes. We do not know for what length of time the worms found in the captured monkeys had been present in the alimentary canal, but it is quite evident from Sandground's findings that they can live for many hours at a temperature in the region of 37°C. which, it may be presumed, is approximately the body temperature of this particular species of monkey. In this connection it is known that the nearly related vinegar eelworm, *Turbatrix aceti*, can tolerate the temperature of the human body since Stiles and Frankland (1902), in reporting its occurrence in human urine, state that the worms were present in the bladder of the patient for a period of 33 days after they were first observed.

Summary.

1. *Cephalobus parasiticus* Sandground, 1939, reported from the stomachs of monkeys, is shown to resemble the sour paste eelworm, *Turbatrix rediviva*, so closely as to be identical with it.

2. It is suggested that the monkeys probably acquired the nematodes by consuming fruit, or some other vegetable substance, on which the worms were living saprophagously ; having been brought to the food by visiting flies.

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Does "Tulip root" in Oats commonly arise from Seed-borne Infection ?

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It has been known for many years that the stem eelworm, *Anguillulina dipsaci*, can be carried in or upon the seeds of a number of different plants. Kühn (1858) originally found the parasite in the diseased seed heads and seed of the teasel or fuller's thistle. Bos (1888) discovered the worms in the flowers of the onion, *Allium Cepa*, and found in one case 3 per cent. of the seeds were infected. Godfrey (1924) found infestations of the parasite in the flower heads of cat's-ear, *Hypochoeris radicata*, and dandelion, *Taraxacum officinale*, and discovered in both hosts that living worms occurred within the seed coats. He also obtained diseased seedlings by sowing infected seed of both hosts. Hodson (1926) reported the occurrence of the parasite in quiescent form beneath the pales of oats harvested from fields badly affected with "tulip root" and obtained small numbers of infected seedlings by sowing such seed. The number of such cases was, however, so small that he considered their occurrence might be accidental. Robertson (1928) investigating "tulip root" in oats, found the parasite in the panicles of affected plants giving rise to abortive flowers. In a number of oat grains from distorted panicles he found small numbers of the parasite between the glumes and pales and between the pales and the kernel and was able to show that after storage for a few months the worms could be revived by soaking the grains in water. He did not pursue his studies to the extent of obtaining diseased seedlings from such infected seed. Cobb (1929) found samples of seed of red clover and lucerne, as cleaned by commercial processes, yielded the parasite in a living condition when soaked in water.

These citations indicate that the parasite may be dispersed by means of infected seed and there is clear evidence in the case of the two Composites, *Hypochoeris radicata* and *Taraxacum officinale*, that the

young seedlings resulting from such seed may show characteristic symptoms of attack by the parasite.

The practical importance of the possibility of the parasite being spread by means of infected oat seed needs no special emphasis, oats being so widely grown a cereal crop. Walton (1938) carried out a series of plot and pot experiments in which he used oat seed obtained from crops which had shown a heavy incidence of " tulip root " but obtained no evidence of infection in the resulting plants. The writer has made a number of observations bearing on the same problem and these are dealt with in the present paper.

Experiments with Seed of Oat *S.82.*

In May 1934 the writer received several oat plants of this variety suffering from " tulip root " which had been grown in variety trials at the Cannington Farm Institute, Somerset. The suggestion was made by the correspondent that possibly the seed was infected though it was recognised that if this had been the case the patchy incidence of the attack and the occurrence of an unaffected area at one end of the field could scarcely be accounted for. A sample of about 2 lbs. of the seed, as sown on the plot, was sent to the writer and with this the following tests were made.

1. Two large sterile Petri dishes were taken and in each were placed 100 seeds which were covered with sterile distilled water. The water was poured off each day for 6 days and examined for the presence of eelworms ; the seeds being covered again with fresh distilled water. No eelworms of any kind were found in the water in which the seeds had been soaked throughout this time.

2. Three Baermann extraction funnels were set up and filled with tap water in which was suspended a muslin bag containing a good handful of the oat seed ; probably numbering well over 1,000 seeds per funnel. Water was run off daily from each funnel into a clean Petri dish and examined for living eelworms and fresh water was added to the funnels to make good that drawn off. Again no eelworms of any kind were found and it was concluded that if any *Anguillulina dipsaci* had been present under the seed coats they could not have been in a viable condition since they did not revive and become motile. The inference was drawn that in all probability the " tulip root " condition observed in the field in this variety was not due to the parasite being seed-borne. It may be

noted that Walton (l.c.) used some of the same seed in one of his trials He sowed 10,000 seeds but failed to find any sign of disease in the resulting crop.

Experiments with Seed of Oat *S.147*.

In June 1938 it was reported to the writer by Petherbridge and Stapley of the School of Agriculture, Cambridge, that oats of the variety *S.147* growing in that district were suffering from " tulip root " and that a seed-borne infection was suspected. A quantity of the seed sown for this crop was sent with the request that the writer would determine the eelworms present inside the glumes. With this material the following trials have been carried out.

1. *Seeds soaked in water.*—A series of samples consisting of some hundreds of seeds were put into Petri dishes and covered with sterile distilled water which was poured off daily and replaced with fresh water. The water in which the seeds had been soaking was examined microscopically but contained no eelworms of any kind during the course of the tests and it was concluded that if eelworms were present under the seed coats they were in a non-viable condition.

2. *Seeds stained.*—Stapley then reported that he had stained a number of seeds in acid fuchsin-lactophenol and had found nematodes beneath the glumes when the latter were removed from the grains and examined microscopically. Later he sent some of his preparations to the writer for examination and identification of the nematodes. On each of four slides the nematodes, seven in all, were found to be *Anguillulina dipsaci* ; five being larval females and two practically adult females. The writer then stained a number of oat grains by boiling for a minute or two in acid fuchsin-lactophenol and after failing to find nematodes in some 30 or 40 seeds, succeeded in finding two examples of *A. dipsaci* (1 male and 1 female) in 2 seeds out of 50 so stained, i.e., approximately 4%.

It was realised that by this technique no information was obtainable as to whether the nematodes found under the glumes were in a living condition. With a view, therefore, to elucidating this point it was decided to grow a considerable number of seedlings and examine each one individually for the presence of nematodes after staining in Scarlet R.

Assuming that roughly 4 or 5% of the seeds were carrying *A. dipsaci* under the seed coats, it was necessary to determine what number of

seeds had to be sown to produce a significant result. It was found that 180 seeds would be adequate to test an infection rate as low as 3%.

3. *Seedlings grown in sand.*—A quantity of silver sand was sieved and thoroughly washed so as to remove large particles and dirt. It was then sterilised by autoclaving and afterwards filled into 180 small cardboard seed cartons which were packed close together in suitable wooden containers. In each carton a single oat seed was planted and sterile distilled water was added. The seeds were sown on June 16th and by June 23rd, 176 oat seedlings were growing well. Ultimately 178 of the 180 seeds germinated and of these 178 seedlings, 1 was definitely poorer in growth than the rest. On July 7th, i.e., after 21 days, all the plants were taken up, the sand was washed from the roots and they were preserved in 70% alcohol in preparation for subsequent staining with Scarlet R. (vide Goodey, 1937).

Of the two seeds which had not germinated, one was found to be quite soft and was without roots or shoot: dissection showed that it contained no nematodes. The other had produced a few small roots and a twisted plumule which, on closer examination, revealed a few brown lesions. No eelworms, however, were found in any part of it after careful dissection. The single poorer seedling of the 178 was dissected but it also contained no eelworms.

The 177 seedlings which had practically all reached the third leaf stage showed no sign of swelling at the base of the stem nor any recognisable symptoms of " tulip root." They were stained in toto in a saturated solution of Scarlet R. in 70% alcohol plus a small proportion of acetone. Each seedling was then washed in plain 70% alcohol and afterwards examined leaf by leaf (each stripped to the base of the stem) in iso-butyl alcohol under the binocular dissecting microscope. The seed coats of the attached seeds were also carefully examined in the same way. Had eelworms been present in the tissue they would have shown up red against the unstained plant tissues since the writer has found this technique very efficient in revealing nematodes in oat seedlings. In not a single plant, however, was a specimen of *A. dipsaci* found. In the case of one seedling 2 eelworms were found, one at the base of the coleoptile and the other inside a glume. These were isolated and on close examination were found to be specimens of *Aphelenchoides parietinus*, 1 male and 1 female.

4. *Seedlings grown in partially sterilised soil.*—Using the same seed of *S.147*, a further test has been carried out during 1939 by sowing 6 rows of seed in a shallow box containing partially sterilised soil. Each row was 28 inches long and the soil was about 4 inches deep. The six rows contained 415 seeds and there was a 100 per cent. germination since 415 seedlings were finally uprooted. After growing for 8 weeks the plants were dug up and each was carefully examined by eye for the presence of any symptoms of " tulip root." In no case was any sign of swelling of the base of the plant found and all of them appeared to be in a perfectly healthy condition.

Conclusion.

It is concluded from these tests that although a few seeds of oats may carry an occasional specimen of *Anguillulina dipsaci* inside the glumes, such worms are not in a viable condition and are incapable of setting up typical symptoms of " tulip root " in oat seedlings. The further inference is drawn that the risk of the spread of the disease by seed-borne infection is probably so slight as to be negligible from the practical point of view.

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Cylindrocorpus nom. nov. for *Cylindrogaster* Goodey, 1927 (Nematoda).

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Perusal of the first volume of "Nomenclator Zoologicus" shows, on p. 927, that the generic name *Cylindrogaster* was first proposed by Stål in 1855 for a Dermapteran insect. As a consequence of this it becomes necessary to put forward a new generic name in place of *Cylindrogaster* which the writer (Goodey, 1927) gave to a saprophagous nematode obtained from a culture of rat faeces.

The name *Cylindrocorpus* is herewith proposed as a nom. nov. for *Cylindrogaster* Goodey, 1927. The new name does not occur in "Nomenclator Zoologicus" and appears, therefore, not to have been used previously as a generic name. It is also satisfactory in that it brings out the cylindrical character of that region of the oesophagus which, in technical descriptions, is called the corpus.

Three species are herewith transferred from *Cylindrogaster* to *Cylindrocorpus* and become :—

1. *Cylindrocorpus longistoma* n. comb. for
   Cylindrogaster longistoma (Stefanski, 1922) Stefanski, 1928
   syn. *Cylindrogaster coprophaga* Goodey, 1927
   syn. *Rhabditis longistoma* Stefanski, 1922.
2. *Cylindrocorpus curzii* n. comb. for
   Cylindrogaster curzii Goodey, 1935.
3. *Cylindrocorpus macrolaimum* n. comb. for
   Cylindrogaster macrolaima (Schneider, 1866) Chitwood, 1933
   syn. *Rhabditis macrolaima* (Schneider, 1866) Oerley, 1886
   syn. *Leptodera macrolaima* Schneider, 1866

The new family name Cylindrocorporidae nom. nov. is also set up in place of Cylindrogasteridae Chitwood, 1933 to include the following genera : *Cylindrocorpus, Goodeyus, Myctolaimus* and *Longibucca.*

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Nematode Parasites of Sheep in Western Australia.

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As yet, apart from a short survey made by Ross (1934) little investigation has been made of parasites of sheep in Western Australia. The present work was carried out, therefore, in a similar manner to that done by Kauzal (1933) in New South Wales, to gain a general impression of the species of parasites occurring here, and also their seasonal incidence.

EXPERIMENTAL MATERIAL AND PROCEDURE.

Altogether a total of 407 sheep was examined, usually at the rate of 10 per week. It was made a practice to select sheep as haphazardly as possible in order to obtain a general sample of wide distribution throughout the State. For periods of three weeks in June and October the number of slaughtered sheep was small and choice was restricted.

The method of estimating the parasitic population was similar to that used by Taylor (1934a). The scrapings from the mucosa and abomasal contents were made up to a volume of 1 litre and the number of worms in a 10 mil. sample determined. The small intestine was treated similarly. Worms in the large intestine were removed by means of forceps.

THE SPECIES OF NEMATODES PARASITISING WESTERN AUSTRALIAN SHEEP.

Altogether 18 different species of nematodes were found. Of these, 15 had been recorded before by Bennetts (1927 and 1935) and Ross (1934).

Diagnosis was first arrived at by use of the key given by Taylor (1935), but later it was found necessary to enlarge it. Of the species noted, those not previously recorded from this State were *Trichostrongylus rugatus*, *Trichostrongylus probulurus* and *Ostertagia mentulata*, the latter never being recorded before in Australia. *Trichostrongylus rugatus* is the only one of this group occurring in numbers of importance.

Ostertagia mentulata was recorded only on two occasions, one being an infection of 200 males, and the other of 100 males, both in the abomasum.

No females of this species were recognised. Baylis (1929) refers to *O. mentulata* as having spicules "remarkably long (up to 0·7 mm.) and conspicuously striated. They terminate in a pair of pincer-like processes about 0·09 mm. long and with hammer shaped or button-like terminations." It was originally recorded from the camel, and Marotel later reported it from sheep in the south of France, but the spicules were found to be only 0·50 to 0·58 mms. in length in the latter case. The spicules in the specimens found in this State were about 0·69 and 0·63 mms. long, the pincers being ·08 mm. in length. The males were about 8 or 9 mms. long.

The Numerical Importance of the Various Species.

Tables I, II and III summarise the results obtained. Little comment is necessary but the prevalence of the genus *Trichostrongylus* is noteworthy. Actually 74% of the sheep examined harboured some parasite of this genus.

Table I.

Forms infesting the abomasum.	Sheep infected %
Haemonchus contortus	13·8
Ostertagia circumcincta	54·7
Ostertagia trifurcata	5·3
Ostertagia ostertagi	Less than 1
Ostertagia mentulata	Less than 1
Trichostrongylus axei	53·3
Forms infesting the small intestine.	
Trichostrongylus colubriformis	53·2
Trichostrongylus vitrinis	40·3
Trichostrongylus rugatus	14·4
Trichostrongylus probolurus	Less than 1
Nematodirus filicollis (chiefly)	31·9
Nematodirus spathiger	
Cooperia curticei	1·3
Cooperia oncophora	1·5
Monodontus trigonocephalum	Less than 1
Strongyloides papillosus	9·4
Forms infesting the large intestine.	
Chabertia ovina	44·9
Oesophagostomum venulosum	Less than 1

Ostertagia circumcincta is of next importance, 18·7% of the sheep having infections of over 500 worms.

TABLE II.

Showing the prevalence of light, moderate and heavy infestations with the various species of *Trichostrongylus*.

Species.	Degree of infestation.	% Occurrence.
Trichostrongylus probolurus...	Light 1 to 500 worms	·3
	Mod. 500 „ 2,000 „	0
	Heavy over 2,000 „	0
	Uninfected	99·7
Trichostrongylus rugatus ...	Light 1 to 500 „	9·6
	Mod. 500 „ 2,000 „	4·8
	Heavy over 2,000 „	·3
	Uninfected	75·1
Trichostrongylus vitrinis ...	Light 1 to 500 „	25·0
	Mod. 500 „ 2,000 „	14·8
	Heavy over 2,000 „	·5
	Uninfected	59·7
Trichostrongylus colubriformis	Light 1 to 500 „	36·0
	Mod. 500 „ 2,000 „	14·7
	Heavy over 2,000 „	2·5
	Uninfected	46·8
Trichostrongylus axei ...	Light 1 to 500 „	36·0
	Mod. 500 „ 2,000 „	14·7
	Heavy over 2,000 „	2·4
	Uninfected	47·0

Though it may appear that *Chabertia ovina* is a frequent parasite of sheep in Western Australia it must be noted that a large number of the infestations were small, only 0·6% being over 500 worms.

In view of its possible relation to " foot-rot " disease (Beveridge, 1934) *Strongyloides papillosus* may be of some importance. Infestations with *Haemonchus contortus* were usually small.

A general indication of the sizes of the infestations recorded is given in Table IV. The highest infestation noted was of 14,000 worms, only two others were over 10,000. As the sheep examined were " fat " sheep sold for slaughter, it is not remarkable that the largest group, 38%, had infestations which could be regarded as moderate in size.

TABLE III.

Showing the prevalence of light, moderate and heavy infestations of the various species of *Ostertagia*.

Species.	Degree of Infestation.	% Occurrence.
Ostertagia mentulata ...	Light 1 to 500 worms	·5
	Mod. 500 „ 2,000 „	0
	Heavy over 2,000 „	0
	Uninfected	99·5
Ostertagia ostertagi	Light 1 to 500 „	·5
	Mod. 500 „ 2,000 „	0
	Heavy over 2,000 „	0
	Uninfected	99·5
Ostertagia trifurcata ...	Light 1 to 500 worms	4·8
	Mod. 500 „ 2,000 „	·5
	Heavy over 2,000 „	0
	Uninfected	94·6
Ostertagia circumcincta ...	Light 1 to 500 „	36·0
	Mod. 500 „ 2,000 „	17·6
	Heavy over 2,000 „	1·1
	Uninfected	45·3

TABLE IV.

Showing the degree of infestation with all species.

Species.	Degree of Infestation.	% Occurrence.
Total parasitic population ...	Uninfected	16·5
	1 to 500 worms	28·0
	500 to 2,000 worms	38·1
	2,000 to 5,000 worms	15·5
	Over 5,000 worms	2·8

THE SEASONAL VARIATION IN THE INCIDENCE OF THE PARASITIC POPULATION.

The months in which maximal average infestations were recorded were May 1936 and March 1937. In the former month the average worm burden was 1,890 worms per sheep and 97·5% of the sheep were infected; in the latter the average worm burden was 1,829 worms per sheep, 97·0% being infected. From May the parasitic population lessened until August, when the worm burden averaged 664 worms per sheep of which 65% were infected. During the months August to

March 1937, there was a steady rise, but during the following month, April 1937, there was a fall to 1,210 worms per host, 90% being infected.

Thus, generally speaking, it can be said that the highest infestations were found during the late summer months February, March, April and May, while the lowest infestations occurred during the winter months of August, September and October.

Attempts were made to correlate the seasonal incidence of the parasites with temperature and rainfall. No close relationship could be demonstrated, but it was found that during the wetter months May, June and July, infestations fell, while during the drier period from August to March infestations steadily rose.

Taylor (1934b) has shown that a good diet is extremely important in combating worm infestation. It seems probable that the better pastures resulting from the increased rainfall during the winter months may have been instrumental in reducing the incidence of the parasites. The winter climate would not appear to be such as to materially decrease worm incidence by preventing larval development. Indeed, it seems more likely that the dry hot summer would be less favourable for development. It is possible that the longer grass and winter weather conditions may have rendered the larvae less accessible to grazing sheep.

The Seasonal Incidence of the Various Species of Parasites.

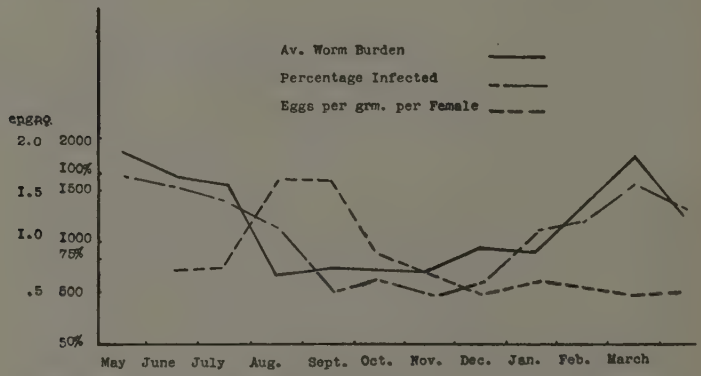
With the exception of *Nematodirus* spp. the seasonal incidence of the different types was similar. The changes were most marked in the case of *Haemonchus contortus*, the average number of this species reaching a maximum of 66 worms per sheep in March and falling to nil in September and November. *Ostertagia circumcincta* was maximal in March, the worm burden averaging 500 per sheep ; the minimum of 57 worms per sheep was recorded in October. *Trichostrongylus colubriformis*, typical of the more common forms of this genus, was most frequent in March, the average reaching 464 worms per sheep. In September this figure fell to 140.

Nematodirus spp. fell in incidence during the period from May to December, after which infestations increased greatly, reaching a maximum of 228 worms per sheep in March. This may have been due to the fact that the temperature did not rise above 24°C. for Cameron (1934) states that hatching does not occur at temperatures below this.

THE SEASONAL CHANGES IN THE EGG OUTPUT OF PARASITIC NEMATODES.

In the hope of acquiring information regarding the factors influencing worm fertility, egg counts were made from the faeces of each sheep examined during this survey. A form of Stoll's method was used.

In the worm counts previously discussed, females and males had been recorded separately, so that it was possible to relate the eggs counted in the faeces to females present in the alimentary tract. The graph shows the seasonal variation in eggs per gram per female, from June 1936 to April 1937. From June to August, when the average parasite population



Showing the seasonal variation in egg output per gram of host faeces per female parasite.

falls steeply, the egg output per female rises steeply. Again, following a rise in the number of parasites present during the months August to March there is a fall in egg output. Thus it would appear that with an increase in the size of the infestation the egg output per female, per gram of hosts' faeces, falls.

Andrews (1936), records that with infestations of *Cooperia curticei* he found that egg production fell with increase in the degree of parasitism. Hill (1926) has reported similar results in the case of hookworms.

In order to bring out the relationship between the change in fertility of the worms and the size of the population, the average egg output for infestations of 100, 200, 300, . . . up to 3,500 worms, was determined.

It was found that the egg output rose steadily until 1,000 parasites were present when 800 eggs per gram of hosts' faeces were noted. From this point egg output per worm decreased, 2,000 worms producing 1,100 eggs per gram and 3,000 parasites 1,300 eggs per gram.

It must be emphasised that these results can only indicate a relationship between the fertility of the worms and the numbers present for the infestations were usually mixed and the egg output of different species of worms varies greatly (Kauzal 1933).

Summary.

1. Eighteen species of nematodes were found to parasitise sheep in Western Australia.

2. *Trichostrongylus rugatus* and *Trichostrongylus probolurus* were found to be present in this State, and specimens of *Ostertagia mentulata*, a species new to Australian records, were found on two occasions.

3. *Trichostrongylus* spp. and *Ostertagia circumcincta* were found to be of chief importance.

4. The seasonal incidence of a number of parasites was found. As a rule, the course of infestations was found to be similar, the highest occurring in late summer and the lowest in mid-winter.

5. A seasonal variation in egg output was recorded, more eggs being shed in the winter months.

6. Results indicated that with increases in the numbers of parasites the egg output per female worm decreased.

Acknowledgments.

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On the Larval Migration of *Syngamus trachea* and its causal relationship to Pneumonia in young birds.

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THE general course of the life history of *Syngamus trachea* has been known for some considerable time. There is a phase outside the body during which the ovum develops to the infective stage. Thereafter it may or may not be ingested by an intermediary. In either event it reaches the definitive host by ingestion with the food. It then migrates to the lungs and thence to the trachea where it settles down to adult life. The stages outside the body are well known but after being taken up by the definitive host, there was a hiatus in our knowledge and the next known stage was the one occurring in the lungs.

Many workers have tried to demonstrate the actual route taken by the larvae from the intestine to the lungs and various theories have been put forward. Walker (1886) suggested that the larvae actually bore through the wall of the fore gut and penetrate the lungs from the outside. Megnin (1883) saw the larvae in the air sacs which he believed were directly connected with the intestine and thought that they had arrived in the lungs *via* these connections. Ortlepp (1923) was unable to find the larvae anywhere in the body between the intestine and the lungs but he believed, as did most other workers, that they penetrated the blood vessels and were carried to the lungs by the blood stream in the way that occurs in other nematodes. Though he made searching examination in all parts of the body he was never able to find the larvae in actual transit. The migration is very rapid for they can be found in the respiratory organs as early as 24 hours after experimental infection.

Recently, in this laboratory, some 6-day-old chickens were fed with *Eisenia foetida* which were carrying infective gapeworm larvae. The earthworms were much more heavily infected than had been realised

and there was a severe mortality among the birds. A number were found dead the following morning, 16 hours later, and many others died during the next few days. The first 2 casualties were closely examined. There were larvae in the lungs where they had caused a severe reaction before death. Blood was drawn off with a fine pipette from the auricles of one bird and in this blood 3 active Syngamus larvae were found. Blood from the postcaval vein of the other bird gave a single larva, also very vigorous and swimming actively in the serum. These larvae had undergone no morphological changes except that they had grown somewhat, measuring now about 0·312–0·395 mm. by 0·017 mm. broad at the widest part. On another occasion 4 other chicks were heavily infected and killed 6 hours later. As much blood as possible was withdrawn from the heart and great vessels and in this a number of larvae were found.

These larvae must have been hatched by the digestive juices probably in the small intestine and have found their way into the blood stream. This stage of active penetration has not however been actually seen and so it is not possible to say whether the larvae have any special predilection for the venous blood or for the lymph. In spite of very assiduous examinations, no larvae were recovered from the liver nor were traces of their presence seen there. However, the occurrence of a single larva in the posterior vena cava is highly significant and they almost certainly pass through this organ in their migrations. They reach the right heart in which situation they have already grown and are extremely active, executing vigorous swimming movements. From the right heart they pass to the lungs where they are filtered out by the capillaries and migrate to the alveoli. At this time they cause considerable damage and may even bring about death. When they break out of the capillaries, haemorrhage occurs and inflammatory processes are set up in the tissues. In the first 2 birds examined the infection was so heavy that the whole of the lungs was ecchymotic and oedematous and the respiratory passages were filled with an exudate which consisted mainly of erythrocytes, leucocytes, some epithelial cells and some fibrin. Most of the lung tissue was consolidated. There was a marked eosinophilia in the tissues. Unfortunately differential leucocyte counts were not made of the peripheral blood. The picture is one of a typical lobar pneumonia. As similar pictures have been recorded elsewhere in various animals infected with other migrating nematode larvae, e.g., *Ascaris lumbricoides* in pigs, we

may use the same terminology and say that these birds had died of ' Syngamus pneumonia.'

From these observations there can be no doubt that *Syngamus trachea* falls into line with many other nematode parasites, e.g., *Ascaris*, *Ancylostoma* and *Heligmosomum* and that the larvae reach the lungs via the blood stream in the usual way.

Another observation which may be of importance in the field was made in the course of these experiments. A large number of partridge and pheasant chicks have in the past been examined post-mortem and have been recorded as dying of ' pneumonia.' This diagnosis has been given largely as a result of macroscopic appearance and the fact that the lungs were heavier than water and consolidated. At the time a number of such lungs were preserved in formalin and these have now been re-examined by teasing and sectioning. Of half a dozen so examined 4 were shown to be heavily infected with nematode larvae. In a few of the sections the larvae showed the typical buccal capsule of *S. trachea*. As the tissue reaction was the same as that already described for the experimental infections the birds may be said to have died of Syngamus pneumonia.

It has long been known that gapeworm causes very severe mortality in the field in its adult stages and it is still one of the major diseases that the game farmer has to contend with. It has not been realised hitherto that young birds in the field may pick up infections heavy enough to cause death during the pulmonary migration. This new factor automatically increases the mortality rate and turns what was only a serious pest into an important menace to young stock. It is interesting to conjecture how many cases of ' death by pneumonia ' in the past are really ' death by Syngamus pneumonia.'

For a variety of reasons the game keeper uses the same land again and again for rearing purposes. It is continuously being reinfected and the snail and earthworm vectors probably get progressively more heavily loaded up with gapeworm larvae year after year. Finally a lethal dose is accumulated and when the snail or earthworm is eaten by a young chicken, the larvae produce symptoms so severe as to cause death. As some of these vectors are capable of surviving up to 10 years, it is easy to understand how massive the vectors' infection may become and how serious may be the consequences to the stock.

Feeding Habits of the Worm.

Syngamus trachea is a blood sucker throughout the whole of its life. In the lungs the worms are surrounded by a copious haemorrhage and continually suck this fluid into the intestine. Erythrocytes can often be seen in the process of digestion. In the adult stage they still keep up the same habits though at first sight the trachea would not seem to be the ideal site for blood-sucking parasites. Digestion is very rapid and it is only rarely that the nature of the gut contents can be recognised. The present writer has been fortunate enough on several occasions to find adult worms, both male and female, in which the buccal cavity contained erythrocytes. These were rapidly drawn down the gut by the very muscular oesophagus and the digestive juices acted immediately. The whole process could be followed under the binocular microscope for the cells became unrecognisable within a few minutes.

Summary.

1. Larvae of *Syngamus trachea* pass from the intestine to the lungs of the definitive host via the blood stream. They have been recovered from blood taken from the auricles of the heart and from the posterior vena cava.

2. The condition of the lungs heavily infected with larvae is described.

3. A condition of ' Syngamus pneumonia ' occurs in the wild state among poults of partridges and pheasants.

4. The worm is a blood sucker throughout its life.

5. *S. trachea* must therefore be recognised as of pathogenic importance in both larval and adult stages.

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Some Polyradiate Specimens of *Taenia pisiformis* and *Dipylidium caninum*, with a bibliography of the abnormalities occurring among Cestodes.

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Abnormalities of structure among the Platyhelmia are by no means rare and so far as the cestodes are concerned were first observed more than two centuries ago. They include various anomalies of segmentation, usually coupled with irregularities in genital development and fenestration which may involve a few segments or the whole strobila. In its most advanced stage fenestration may result in the appearance of two strobilae when the internal organs are divided between the two sets of segments. This condition resembles superficially a dichotomous division of the strobila, which has been reported from time to time. A single scolex has attached to it two strobilae but in the case of dichotomy, each segment in each chain has a complete set of internal organs. A single case of fusion has been recorded. Here the strobila formed a closed loop. Pigmentation occurs in cestodes: it may have a natural geminal origin or it may be due to the absorption of drugs or haemoglobin.

A completely different type of abnormality gives rise to the polyradiate specimen. The worms may be tri-, quadri- or penta-radiate and such a condition is always associated with multiplication in the number of suckers and sometimes of rostella. It is generally considered to be due to an incomplete division in the ovum in its early stages giving rise to a kind of Siamese twin. Such abnormal hexacanth embryos with twice the number of larval hooks and also cysticerci with the typical polyradiate scolex have been described for a number of species.

We may consider each kind of abnormality briefly. Irregular segmentation may be incomplete, the partition reaching only part way across the segment after which it peters out. Or the division may be oblique and

meet the preceding division resulting in the interpolation of a small triangular segment. Both these conditions are exceedingly common and have been recorded for a number of species, particularly *Taenia saginata* and *Dibothriocephalus latus*. Spiral segmentation has been recorded several times—in *T. saginata* and *Moniezia expansa* among others. Here the segmentation on one side of the worm joins up with that of an earlier segment on the other side, giving a spiral effect. Both incomplete and spiral segmentation have been seen in this laboratory in a specimen of *Hymenolepis farciminosa*, a starling parasite. Occasionally extra divisions will be interpolated and in a few cases it has been reported that segmentation has completely disappeared with the result that the genitalia are repeated more or less regularly without any lines of demarcation. Such irregularities have been recorded not only in the species already mentioned but also in "a parasite of gallinaceous birds," probably *Choanotaenia infundibulum*, *Taenia pisiformis*, *T. solium* and *Dibothriocephalus pythonis*.

Frequently associated with these anomalies in segmentation are disturbances of the genitalia. Intercalary segments may be quite sterile or may contain an entire set of glands or a portion—perhaps an ovary or a cirrus sac. The genital pore is often anomalous and extra ones appear which may or may not be connected within the glands. Bork, for instance, cites a case when one segment of *Taenia solium* was provided with 22 genital pores and Grobben reports on a "segment" of *T. saginata* 128 mm. long which had 41 genital pores. The genital glands were rudimentary.

Reduction in the genital glands is very frequent. It may be partial, involving perhaps an ovary or any other organ or it may be complete. Diamare and Shipley have each noted the disappearance of a set of organs in *Dipylidium caninum*. On the other hand duplication in whole or part is also known and has been recorded in *Taenia saginata*, *T. solium*, *Hymenolepis diminuta*, *Ctenotaenia goezei* (*Cittotaenia denticulatum*), *Moniezia expansa*, *D. caninum*, *Dibothriocephalus latus*, and *Diplogonoporus sp.* Nitzulescu reports a case of complete inversion of organs in *T. saginata* and similar records are on hand for other species. The testes in *Hymenolepis*, usually 3 in number, have in certain species been increased to 4 or decreased to 2. Both conditions have been recorded in *H. nana* and *H. fraterna*.

Melanism is not unknown among the cestodes. Pigment almost normally occurs in the scolex of *T. saginata* but there are on record a number of cases where the pigment is distributed throughout the whole strobila. It is reported also in T. *solium*. There are many records of pigmentation but in some cases it is thought to have been caused by the administration of a drug to the host and its later absorption by the parasite for it has disappeared when such treatment has been discontinued. Oelkers records *T. saginata* with a grey colour which was due to the absorption of mercury salts with which the host, a syphilitic patient, had been treated. It is interesting to note that the helminth seemed to be completely unaffected and was very vigorous. Redon records the presence of iron salts in cestodes giving rise to apparent melanism while Railliet describes *Anoplocephala cuniculi* (*Cittotaenia pectinata*) of a rosy colour caused by the absorption of haemoglobin. The worm had been bathed in a copious haemorrhage for some time before expulsion, the host having been heavily parasitized with *Trichostrongylus retortaeformis*.

A case of partial melanism was observed in this laboratory in the species *Taenia pisiformis*. A single specimen, among 16 others, had a deposit of black pigment in every segment, in the vas deferens and cirrus sac.

Fenestration has been recorded from *T. saginata*, *T. solium*, *T. marmotae* (*Cittotaenia pectinata*) and *Dibothriocephalus latus*. It may affect only a single segment, a short chain or the whole strobila. Usually, but by no means always, the genitalia in such segments tends to be reduced.

Dichotomy has occurred many times in the following species—*D. latus*, *D. microcephalus*, *D. proboscideum*, *D. hians*, *Tetrarhynchus bisulcatum*, *Solenophorus megalocephalus*, *T. hydatigena*, *T. crassicollis*, *T. saginata*, *Chapmania tauricollis*, *Anomotaenia sp.* and *Peltidocotyle rugosae*. Usually the dichotomy was limited to a few segments but complete bifurcation beginning close behind the head has been recorded several times and most recently perhaps by Chandler for *T. pisiformis*. In *Trigla gurnardus* larva van Beneden describes a specimen with a normal scolex but the attached bladder was double. This might possibly have developed into a double monstrosity in the adult. In some cases dichotomy has not been limited to a single division but the branches themselves have divided, giving the final appearance of several ragged tails.

Bork records the development in two specimens of *Ligula* of buds of varying lengths, which were striated like the parent part of the worm but he does not give details of the anatomy.

The hook crown sometimes shows unusual points. The number and form may be reduced, and Delore, Cobbold and Weinland have all recorded the presence of a triple crown of hooks in *Cysticercus cellulosae*.

The shape of the segment is often a little variable. Specimens of *D. latus* for instance may have segments of Taenioid shape.

A completely different type of malformation which is inherent in the make-up of the worm leads to the so-called polyradiate forms of cestode. By far the most frequent type is the tri-radiate and this has been recorded in *T. solium*, *T. saginata*, *T. crassicollis*, *T. pisiformis*, *T. hydatigena*, *Multiceps multiceps*, *Echinococcus granulosus*, *Dipylidium caninum*, *Hymenolepis megalöon*, *Dibothriocephalus tectus*, *Anoplocephala perfoliata*, and *Triaenophorus nodulosus*. This condition is always associated with the presence of 6 suckers on the scolex and occasionally with 2 rostella. Quadri-radiate forms with 8 suckers and 4 flanges have been recorded for *T. saginata* and *T. balaniceps* and a penta-radiate form of *T. saginata* had 10 suckers and 5 wings to the strobila. Such forms are now recognised to be a case of incomplete twinning and the larvae which give rise to them have been described by a number of people. Six-suckered cysticerci have been recorded for *C. cellulosae*, *C. bovis*, *C. fasciolaris*, *C. pisiformis*, *C. tenuicollis*, *Coenurus cerebralis*, and *C. serialis*. Most of these forms have a single rostellum but Leiper in 1913 described a *C. pisiformis* with 6 suckers and two rostella.

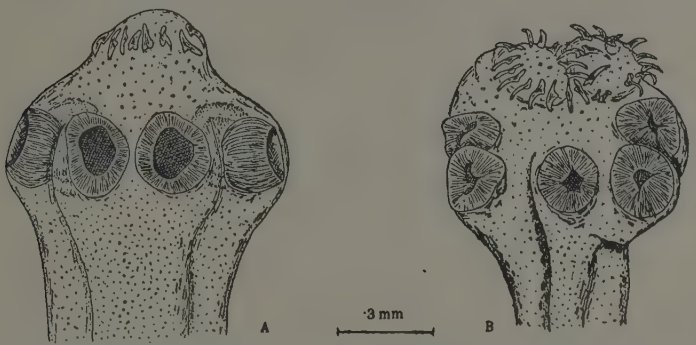
Railliet described a large number of scolices of *C. serialis* which showed great abnormalities in the number of suckers ; they varied from 3 to 10. It is difficult to imagine into what kinds of adults some of these would have developed. Some had one rostellum, others had two. Rudolphi described a *Cysticercus tenuicollis* which had two scolices attached to one bladder.

To take the development of polyradiate cestodes further back, we have records of onchospheres with a double number of hooks which presumably would have developed into the abnormal cysticercus. There are a number of records of this condition for *T. saginata*, and Davaine records a 12-hooked embryo in a bird cestode which was probably *Choanotaenia infundibulum* while Salzmann describes a similar form with 12 hooks for *D. caninum*.

It will be seen that abnormalities among cestodes are extremely common and of the species involved, *Taenia saginata* seems to be very prone to the formation of anomalous forms. Many polyradiate forms were at first described as new species before they were recognised at their true value. *T. saginata* for instance has been *T. lophosoma*, " T. from the Cape of Good Hope," *T. mediocanellata*, etc.

Description of the Polyradiate Forms.

Recently in this laboratory a number of polyradiate specimens of *T. pisiformis* and a single specimen of *D. caninum* have been obtained from local dogs and in view of their frequency and variety, their presence



Figs. A & B show tri-radiate heads of *T. pisiformis*, one with a single rostellum and the other with two. In both, reduction of the hook development can be seen.

is here recorded. Of 159 dogs examined, 39 were infected with *T. pisiformis* and 3 of them harboured polyradiate forms. An aged fox terrier bitch weighing 13 pounds harboured over 400 cestodes, 320 of which were *T. pisiformis*. Among this great bulk were 8 tri-radiate specimens. One had 2 rostella, the others all had a single one. Another dog autopsied the same day had a single specimen of *T. pisiformis*—a young one with the bladder segment still attached. This was tri-radiate and the scolex had one rostellum and 6 suckers. The third dog was a male mongrel, weighing 44 lbs. It contained a few segments of *D. caninum*, 140 normal, 10 tri-radiate and 3 tetra-radiate *T. pisiformis*. They had a single rostellum except one of the quadri-radiate forms. They were allowed to die relaxed in water and before fixation they gave the following measurements.

(*A*) *From the first dog.*

1.	2·2 cms.	containing	57	segments.
2.	11·0 ,,	,,	101	,,
3.	13·8 ,,	,,	93	,,
4.	19·4 ,,	,,	132	,,
5.	19·5 ,,	,,	130	,,
6.	19·7 ,,	,,	130	,,
7.	22·1 ,,	,,	134	,, (this worm had the double rostella)
8.	30·5 ,,	,,	144	,,

(*B*) *From the second dog.*

1.	4·7 cm.	containing	74	segments.

(*C*) *From the third dog.* 1–9 being tri-radiate, 10–12 quadri-radiate.

1.	7·3 cms.	containing	92	segments.
2.	8·5 ,,	,,	97	,,
3.	10·8 ,,	,,	102	,,
4.	11·0 ,,	,,	102	,,
5.	11·1 ,,	,,	99	,,
6.	11·2 ,,	,,	105	,,
7.	12·0 ,,	,,	113	,,
8.	13·2 ,,	,,	111	,,
9.	13·5 ,,	,,	126	,,
10.	7·5 ,,	,,	90	,,
11.	7·6 ,,	,,	98	,,
12.	8·1 ,,	,,	92	,, (this had 2 rudimentary rostella)

None of these worms were gravid though normal worms of similar length contained ripe eggs in a well-developed uterus. In these worms the uterus could be seen developing. There were a few abnormalities in the strobila. Segmentation was occasionally irregular. A number of intercalary segments were present, involving 1 or 2 wings. These were usually sterile but two had a few testes and a genital pore which was not however functional. In another segment there was almost complete doubling of the organs. There were 2 vaginae, 2 cirrus sacs and 2 pores but the ovary was simple, though large. In a single case 2 segments had fused ; there were 2 sets of genitalia and 2 pores, regularly arranged one behind the other.

In the tri-radiate forms the genital pores occurred on any of the three edges of the strobila and no single edge seemed to be more frequented than the others. Some segments had 2 pores, others a single one and two had no pore. There have been records, e.g., Cobbold, of the pores all occurring on the same wing.

The genital glands all occupied all three wings equally and thus the ovary and yolk glands came to be tri- instead of bilobed. The unpaired shell gland was situated at the joint of section of the wings. As unfortunately none were gravid it is impossible to say what form the uterus would assume or what the eggs would be like. There were 3 longitudinal excretory canals, but the connecting canals were difficult to make out and seemed to follow no general order.

The first rudiments of the genitalia appeared early in segments 6–8 and the lateral projection due to form the cirrus sac about the 26–28 segments. The rudiments of the testes appeared about segment 65 and were functional about 90–103, at about the same time as the first well formed penis and pore appeared. The ovary began to appear about segment 90 and was functional from segment 110–115 onwards. In the longest worms could be seen the first outpushings of the uterus as a simple tube.

Normal worms of similar length were further developed and gravid; the terminal segments contained uteri with mature eggs. Few of the worms were very long: this may have been due to the great bulk of parasitic material in such a small animal.

The flanges in all the worms were continued up the unsegmented neck to the scolex itself, terminating at the level of the suckers, so that these organs appeared in pairs on a well defined ridge. There was nothing abnormal in the appearance of the suckers except that they were much smaller but certain changes had occurred on the rostellum. The hooks were often of a typical form, particularly the large ones, but there were a few malformations among the small ones, usually in the spine. This was reduced to a variable degree. In some it resulted only in a blunting of the tip but in others it had almost disappeared and could be seen only as a rounded knob. On the whole however, the hooks were easily recognisable as Taenia hooks. The size varied from 103μ–159μ long in the small ones while the large ones were from 230μ–240μ long. The number in each circlet varied from 16–20. The size of the rostellum was reduced. Normal worms from this animal gave a measurement averaging 450μ in diameter with but little variation. The abnormal ones measured from 300μ–350μ and in the worm that had the two rostella the sizes were further reduced to 210μ and 220μ. The suckers measured only about 210μ in diameter, the largest being 240μ while normal suckers measured close on 400μ.

The quadri-radiate forms showed no extra abnormality except a simple intercalary segment involving one wing. The wings were not always of the same size and disposition. In one worm a cross section gave the appearance of a maltese cross, each pair of wings forming a right angle. In the other two worms, two of the wings were small and folded back so that the cross section was a letter " H " with one upright shorter than the other. The genitalia invaded all the arms and appeared to develop normally. The pores were spread over all four wings. Most of the segments had two.

The scolices were not similar in these forms. All had 8 suckers, arranged regularly in 4 pairs, normal in appearance and form except for a reduction in size. They varied from 220μ–275μ in diameter. Two worms had a single well marked rostellum, complete with 2 crowns of hooks. The diameter was 310μ and therefore small. The third worm had a very unusual scolex and indeed identification was based solely on the fact that all the other taenioid worms in this dog were *T. pisiformis*. There were a pair of slightly muscular patches indicating where two rostella might have been expected. There were no hooks.

Tri-radiate segments of *Dipylidium caninum* were also recovered from a dog, a young Retriever weighing 28 lbs. The material consisted of 13 segments. There were short chains of 7, 3 and 2 segments and an odd one. All were gravid. The scolex and mature segments were not found and the whole was rather decomposed. The three wings were not of equal size, one being narrow. The two wide wings met at a very acute angle and lay one on top of the other, so that the superficial appearance was that of a ribbon, thickened at one side. These two wings were typical of the worm, containing egg nests and each had a genital pore with vagina and vas deferens remaining. The narrow wing also had egg nests and in most of the segments there was a pore which was perforate and functional. In one segment however these structures were only vestigial : the pore was not open and the ducts not properly differentiated. There were no intercalary segments. The onchospheres were normal with 6 hooks each.

Abnormalities among *T. pisiformis* must be of very frequent occurrence as they have been found among 8% of the infections seen in this laboratory in the space of a year. The dogs were all domestic dogs, sent to a local veterinary surgeon for destruction and may be said to represent a typical cross section of dogs in a semi-rural area.

SUMMARY.

(1) A brief summary of abnormalities among Cestodes is given.

(2) A new record of melanism in *T. pisiformis* is made. The pigment was deposited in the vas deferens and cirrus sac in every segment.

(3) 18 specimens of tri-radiate and 3 specimens of quadri-radiate *T. pisiformis* are described from dogs.

(4) A single case of tri-radiate *D. caninum* is reported.

(5) A bibliography containing records of cestode abnormalities is appended.

I am indebted to Dr. R. T. Leiper for giving me the use of his library in which I was able to examine many of the earliest publications on this subject and to many libraries in London who have generously allowed me to search their literature.

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The Occurrence of *Heterakis gallinae* in Poultry and its Relation to Disease, Breed, and to other Helminths.

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In a recent paper (Morgan & Wilson, 1938) the authors gave an account of a general survey of helminths in poultry in Scotland and pointed out that *Heterakis gallinae* was by far the most common species occurring in this host and that 82·3% of the birds examined were infected. Not only was the incidence high, but heavy infestations amounting to hundreds of worms were frequently found in these birds which had picked up the worms under natural conditions.

It is somewhat surprising, therefore, that the attempts which have been made to build up heavy infestations of *H. gallinae* in birds under experimental conditions have not, so far, been successful. Clapham (1934) found that only about 100 to 150 worms can be induced to develop after artificial feeding and that a dose of 300 embryonated eggs gave the highest percentage of worms (39·3%) developing in the host. Doses of 1,000 eggs gave a percentage survival of only 0·3. Clapham concludes " that it seems impossible to build up heavy infestations of *H. gallinae* in the chicken," and that " while it is difficult to show a definite immunity reaction on the part of the host, yet only a small percentage of eggs reaches maturity in the host."

It seems evident that fowls under natural conditions are exposed to some factor or factors which tend to break down this partial immunity to heavy infestations which is so marked in experimental birds. It is possible that certain diseases may lower the resistance of fowls to helminths, although the authors found no evidence during the general survey mentioned above that any of the common diseases had this effect.

In fact, the presence of tuberculosis seemed to be very unfavourable to the development of worms generally. Clapham (1938), on the other hand, found that leukaemia was associated with very heavy helminth infestations and concludes from the presence of large numbers of young stages that many more larvae than normal were able to develop during the course of the disease.

It seems clear, however, that disease is not the only factor influencing the development of *H. gallinae*, since healthy fowls have frequently been observed to harbour large numbers of this species. This was shown during the examination of 170 young cockerels which had all been reared on the same farm and had been killed for market. Over 46% of these birds harboured over 100 specimens of *H. gallinae* and an appreciable number showed infestations ranging from 200 to 500 worms, figures which are well beyond those obtained in experimental birds.

The observations recorded in this paper were made during the examination of 230 diseased birds from a flock of hens which were kept on a poultry farm while undergoing egg-laying tests. The birds varied in age from 6 to 18 months, but the majority of deaths occurred between the age of 10 and 16 months and coincided roughly with the time of greatest egg production by the fowls and also of optimum conditions for the development of the eggs and larvae of helminth parasites. It was, therefore, possible to gain some idea of the worm burden of fowls under conditions which might be considered favourable for the development of heavy infestations, and as all the birds were obtained from one source it was thought that the chances of picking up worm eggs and larvae would not vary to any great extent. Furthermore, since heavy-laying strains from several of the common breeds of fowls were used in the egg-laying test observations could be made on the possible relation of helminth infestations to breed.

Incidence and Intensity of the Infestations in the Present Series of Observations.

A comparison between the results obtained in the present series and those obtained during the general survey of helminths in Scottish poultry is shown in the table on p. 179.

It will be seen that in most cases the worm infestations were more frequent and reached a higher level in the present series of birds than in those of the general survey. This marked difference might be attributed

	Present Series.	General Survey.
Percentage infected with *H. gallinae*	86·6	82·3
Average number of *H. gallinae* per infected bird	206·1	99·1
Percentage of *H. gallinae* infected birds with over 100 worms....	52·1	26·4
Percentage infected with *Capillaria* spp....	53·3	43·0
Percentage heavily infected with *Capillaria* spp.	28·8	29·4
Percentage infected with *Davainea proglottina*	33·5	14·9
Percentage heavily infected with *Davainea proglottina*... ...	13·0	—
Percentage infected with *Ascaridia galli*	27·4	11·1

to a lowered resistance to worms in a flock of heavy-layers, but this is difficult to decide, since the birds examined during the general survey were drawn from many different centres and the chances of picking worms would therefore vary very considerably. This would tend to give lower figures than would be obtained in birds from one farm where the ground had become heavily contaminated as the result of carrying fowls for many years. This was shown in our previous paper (1938) where the counts obtained from two farms are shown to be generally higher in each case than those of the general survey. It is doubtful, therefore, whether the heavy infestations observed in the present series can be attributed to a loss of resistance to helminths during a period of heavy egg production or to any exceptional contamination of the runs on which the birds were kept.

H. gallinae *in relation to disease.*

In order to study the possible influence of disease on the development of large numbers of *H. gallinae*, the following grouping of the diseases observed in the birds of the present series was adopted :—diseases of the reproductive system, diseases of the alimentary canal, fowl paralysis, tuberculosis, nephritis and visceral gout, miscellaneous.

Diseases of the reproductive system include inflammation and impaction of the oviduct, often terminating in peritonitis, prolapse, broken egg in the oviduct, egg-binding, etc., and diseases of the alimentary canal include impaction of the crop, gizzard and intestines, enteritis, intussusception and cloacitis or vent gleet.

Under miscellaneous are classed those conditions which occurred infrequently, such as fowl pox, contagious catarrh, pneumomycosis, fatty degeneration of the liver, tumours, etc.

In the following table is shown the average number of *H. gallinae* per bird for each of the disease groups :

	Number Cases.	Average *H. gallinae* per bird.
Fowl paralysis	48	165·4
Diseases of the reproductive system	42	232·0
Nephritis and Visceral gout	37	200·8
Tuberculosis	22	50·2
Diseases of the alimentary canal	21	209·6
Miscellaneous	45	197·2

The average number of *H. gallinae* per bird in all the disease groups was 183·7.

The above table shows that the level of infestation with *H. gallinae* is very low in the birds suffering from tuberculosis as compared with that in the other disease groups. This confirms a previous observation made by the authors (1938), where it was noted that out of 53 tuberculosis cases only 6 had even a moderately heavy infestation. It was also noted in the birds of the present series that where a fair number of worms was present the disease was invariably at an early stage.

It was found from a statistical analysis of the figures given for the disease groups that tuberculosis is associated with a notably smaller degree of infestation than the other diseases, and that the other diseases do not significantly differ from each other. The suggested association between fowl paralysis and heavy infestations of worms receives no support from the data given in the above table; in fact, the figure given for this disease is, with the exception of tuberculosis, the lowest in the series. This confirms the observations reported in our previous paper (1938).

Only 6 of the birds examined were found to be suffering from leukaemia and the number is therefore too small for comparisons to be made with other diseases. It was found, however, that three of these birds harboured *exceptionally* heavy infestations of *H. gallinae*, a finding in accord with that of Clapham (1938), who considered that this disease lowered the resistance of the birds to helminths.

It is of interest to note that in spite of the heavy infestations with worms found in the birds in the present series of observations only in three of the cases was the cause of death attributed to worms; a diagnosis based on the finding of a large number of worms, emaciation in the host and absence of specific diseases. In the birds so diagnosed the different species of *Capillaria* were, in particular, abundantly

represented in addition to other forms. The fact that only in three cases could death be attributed to worm infestation is at variance with results obtained at certain other Egg-Laying Tests. In some of these the death rate due to worms appears to be very high, and has in one instance been recorded at 40 per cent.

The belief is very general that much of the loss at present experienced in the poultry industry is due to helminthic infestation. This is not supported by our observations, and it is our experience that even large numbers of worms are not diagnostic of helminthic disease unless associated with the appropriate pathological symptoms.

H. gallinae *in relation to breed of fowls.*

As several of the common breeds of fowls were undergoing egg-laying tests under the same conditions of housing and feeding it was possible to determine whether these breeds varied in their susceptibility to *H. gallinae.* Three of the breeds, viz., White Wyandotte, White Leghorn and Rhode Island Red, being the most common, provided a sufficient number of cases for comparisons to be made.

The following gives the average number of *H. gallinae* per bird for each of these breeds :

White Wyandotte	...	...	...	118·7
White Leghorn	...	...	...	216·6
Rhode Island Red	...	...	...	189·3

These figures would seem to indicate that the White Wyandotte is less susceptible to *H. gallinae* than the other two breeds, and analysis of the detailed figures showed a markedly lower infestation in the White Wyandotte than in the White Leghorn, and also that the difference between the White Wyandotte and the Rhode Island Red was almost significant, the probability in the latter case being slightly greater than 1 in 20.

H. gallinae *in relation to other helminths.*

It was thought that one of the factors influencing the development of *H. gallinae* in numbers higher than are obtained in experimental birds might be the presence, in birds under natural conditions, of other species of worms. In order to test this possible relation of *H. gallinae* to other helminths the birds in this series were divided into two groups ; those showing *heavy* and those showing *light* infestations with one or more

species other than *H. gallinae*. The average number of *H. gallinae* per bird in these two groups was as follows :

	Heavy Group.	Light Group.
Number of cases	61	154
Average *H. gallinae* per bird ...	284·6±40	146·5±18

Statistical analysis of the detailed figures obtained in this series of observations showed that the difference between these two groups is highly significant and the chance that it could arise from randomness is less than 1 in 400. The samples significantly differ also, not only in their means, but in their variabilities, the birds in the *heavy* group having much the greater mean and greater variability.

This relationship of *H. gallinae* to infestations with other worms is not apparently influenced by the fact that the birds in this investigation were suffering from disease, since the same marked difference was obtained in the group of 170 healthy birds mentioned above. In these healthy birds the average number of *H. gallinae* in the group heavily infested with other worms was 291·5 as compared with 116·1 in the lightly infested group. This difference is even wider than that obtained in the present series.

It is not suggested, however, that the presence of other worms is the only factor influencing the development of large numbers of *H. gallinae*, as individual birds are frequently observed with large numbers of this species and a complete absence of other worms. Other factors such as individual susceptibility, disease and breed of fowl may all play a part, although the evidence at present seems to point to the presence of other worms as being the most important factor associated with the development of *H. gallinae*.

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The Structure of the Leaf Galls of *Plantago lanceolata* L. induced by *Anguillulina dipsaci* (Kühn) Gerv. & v. Ben.

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Introduction.

This investigation was carried out for two reasons, firstly that there would appear to be no detailed description of the changes in the leaf structure in *Plantago lanceolata* L. under the influence of parasitization by *Anguillulina dipsaci* (Kühn) Gerv. & v. Ben., and secondly that material for study was readily available.

Anguillulina dipsaci attacks a large number of plants, the latest unpublished list containing the names of 285 hosts. Among them are three species of Plantago. The first record of nematode galls on *P. lanceolata* is, according to Houard (1908), one by Liebel in 1886, when the galls were attributed to *Tylenchus* sp. Ritzema Bos (1888–92) showed that the infecting agent was *A. dipsaci*. In Britain the first record is by Hodson in 1929 when he found galls on *P. lanceolata* due to *A. dipsaci* at a number of localities in South Devon. At the same time he recorded infection on *P. major* L., *P. maritima* L., and also on *Hypochaeris radicata* L.

The material forming the subject of the present study was obtained from an infected plot at Winches Farm, St. Albans, Herts. The original source of infected plants was No-mans-Land Common, near Wheathampstead, Herts; where they were discovered by Dr. A. Smith of the Ministry of Agriculture Plant Pathological Laboratory, Harpenden. This locality thus constitutes another record of infection. The fact that there are so few records of parasitization is in all likelihood due to failure to find them and not to the absence of infection.

Methods.—The infested leaves were pickled immediately after gathering, using two fixatives, Bouin's fluid and a modification containing urea.

In addition to this some material pickled some time previously in Acetic Alcohol was used. Both normal and galled leaves were fixed, the galled ones showing various stages of parasitization. Material was examined both macro- and microscopically, in the fresh and pickled conditions. Hand and microtome sections were cut; embedding was carried out using the normal ethyl-alcohol method. Various combinations of stains were used; Safranin and Haematoxylin, Safranin and Light Green, Gentian Violet and Light Green, and Gentian Violet and Orange G, all of which had their value.

Normal Leaf Structure.—The uninfected leaf (Fig. 1) exhibits comparatively normal dorsi-ventral structure. The underside shows five ridges below the five main vascular bundles. The upper and lower epidermides are simple and each have stomata. There are numerous hairs on the leaf, mostly towards the proximal end. A stomate is flanked by a pair of subsidiary cells placed transversely to the aperture of the stomatal pore. The mesophyll is divisible into palisade and spongy parenchyma, the palisade being about two cells deep, and the spongy tissue being composed of roughly iso-diametric cells. The intercellular space system is, in volume, in keeping with the comparative lack of differentiation shown by the mesophyll.

The vascular bundles are normal collateral ones and there are two patches of collenchyma above and below the large bundles. The smaller bundles have no collenchyma. An endodermis surrounds the bundle, and there is a Casparian strip on the radial cell walls though it is by no means distinct.

The Galled Leaf.—The infected plant has a blotchy diseased appearance, the usual regular rosette being disfigured by the twisted and displaced leaves. The gall is not so much a definite morphological entity, as a swollen portion of the leaf. A slightly infected plant may appear just to have a few pale greenish patches, but the more heavily attacked show all stages of swelling, from the slight pale green areas through whitish to brown badly swollen galls; one of the effects of infestation is an apparent loss in the amount of chlorophyll consequent with the gradual death of the cells of the gall.

In lightly infected plants, the galls tend to occur between the veins of the leaf, but in heavy attacks the whole width of the leaf becomes involved, so that the veins lose their identity as ridges and become part

of the general swelling. The lower epidermis of such a gall is pale green or brownish and shows a somewhat puckered appearance.

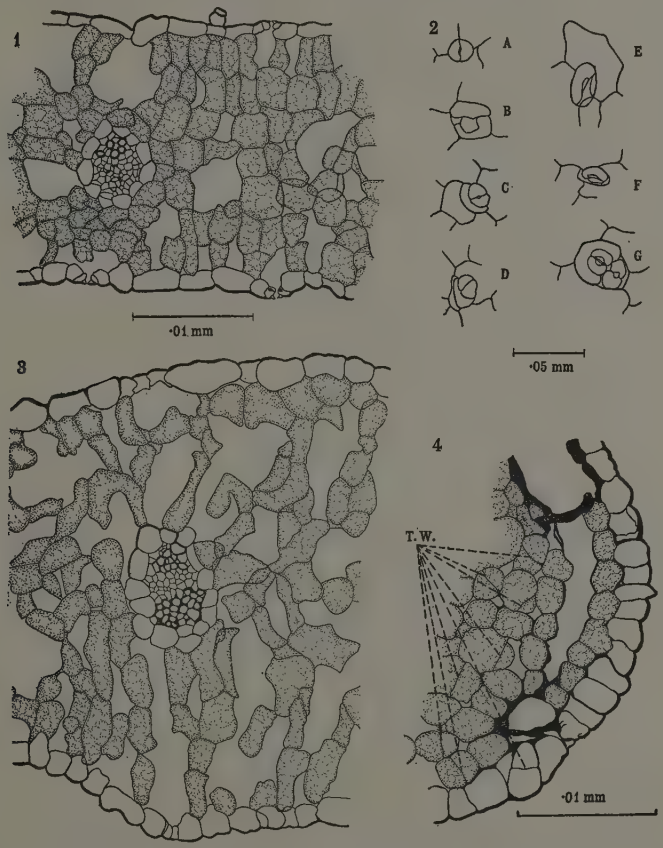

Fig. 1.—Transverse section, young normal leaf of *Plantago lanceolata*.

Fig. 2.—(*a*) Normal stomate, (*b–g*) Abnormal stomata from epidermides of galls.

Fig. 3.—Transverse section, young galled leaf of similar age to that in Fig. 1.

Fig. 4.—Transverse section of part of a galled leaf, showing cell division by tangential walls cutting off a necrotic area.

The stomata (Fig. 2) in both upper and lower epidermides show many abnormalities. Various types have been observed; for instance, in one

a stomatal initial cell had divided into three and the pore was rudimentary and unopened, in another even the rudiments of a pore were missing. Others have one guard-cell more developed than the other, giving a lop-sided appearance. Not only do the guard-cells show modifications but the subsidiary cells also may be abnormal. Here may be found, for instance, a triplet of subsidiary cells, which probably means that one of the normal pair has divided; still further cases occur in which the stomate is surrounded by a number of cells, so that the individuality of the subsidiary cells is lost. In another case one subsidiary cell practically surrounded the stomate, the other being very much reduced in size. In addition to these separate modifications, combinations of the two states are also found giving quite a considerable variation of stomatal abnormality.

It is, however, the mesophyll of the leaf which undergoes most change due to parasitization. Where the infection is heavy the differentiation into palisade and spongy mesophyll is lost, and though the palisade region tends to be of more closely packed cells, it is of similar type to the rest of the mesophyll. In the spongy parenchyma region the cells are considerably enlarged, and while in some cases they tend to become cylindrical and arranged in rows radiating below the vascular bundle, in others they take on a more spherical form and, where contiguous with one another have bulges giving the appearance of short stumpy pegs.

The vascular bundles show certain changes especially the larger ones that are contained within a gall. The main change is an increase in girth, the amounts of both xylem and phloem being greater, and there are signs of a certain amount of cambial activity having taken place. The collenchymatous patches above and below the bundles are greater in amount. In the smaller leaf bundles there is normally no collenchyma present, but in those in the galled leaf (Fig. 3) a patch is usually found below the vascular tissue. The endodermis surrounding the bundles, although in small youngish galls shows a Casparian strip, in old ones shows no trace of it whatever.

In lightly infected youngish leaves, in which the nematodes responsible are confined to a few places, there is a tendency for some cambial activity to occur (Fig. 4), and some of the cells surrounding the parasitized area divide tangentially, so localizing the infection.

The intercellular space system in the mesophyll is considerable, for the cells, by their enlargement, tend to break away from one another.

Not only is cell hypertrophy to be found, but also proliferation, thus giving rise to the large swelling of the gall. In conjunction with hypertrophy, the phenomenon of multinucleate cells is met, though only in a few of the hypertrophied cells. The presence of binucleate cells in normal tissue seems to be an event of not infrequent occurrence, but in *P. lanceolata* it appears to be confined to the hypertrophied cells of the mesophyll.

The nematodes are located in the intracellular spaces and here they live and reproduce, for both worms and eggs are to be found. When the gall eventually decays and rots away, the worms are liberated and can then infect other plants.

In the galls of *P. lanceolata* numerous areas of collapsed and semi-collapsed cells are found, their close proximity to the nematodes indicating the position or late position of the parasite. These cells contain numerous granules and both they and the affected cell walls always take stains very deeply. In the fresh condition the collapsed walls are brown in colour. Granules are also to be seen in whole cells contiguous with the necrotic patches. The granules seem to be of the nature of proteins, since both they and collapsed cell walls give positive reactions to the Xanthoproteic test, and also to Millon's reagent. To the Biuret test no observable reactions resulted. Treatment of sections of the galls with dilute Iodine showed the collapsed cells stained brown, and both the granules and the nematodes yellow. Starch grains in non-collapsed cells stained pale mauve, whilst those in cells further away from the focus of infection stained more deeply.

Discussion.

Goodey (1935) discussing the aetiology of plant lesions says that two ways have been recognised by which the parasite can injure the plant, namely mechanically and chemically. Both he and Kostoff & Kendall (1929) accept the hypothesis that injury is chemical, and the evidence of Beyerinck (1883) and of Ritzema Bos (l.c.) supports this view strongly. The work of Quanjer (1927) also supports the chemical hypothesis, and further he shows that there is a dissolving action, by the substances emanating from the nematodes, upon the middle lamella.

Goodey (l.c. p. 19) expresses the opinion that though *A. dipsaci* and other nematodes possess stylets, they do not use them in any way to puncture cells. He does, however, say that while there is no evidence

either way, an open mind must be preserved upon the subject. Since then Linford (1937 a, b) and Linford & Oliveira (1937) have shown that nematodes, and among them *A. dipsaci*, not only puncture cells by means of the stylet, but also feed upon their contents.

The presence of collapsed and semi-collapsed cells in the galls on *P. lanceolata* is, in all probability, due to this feeding mechanism of *A. dipsaci*, and though it has not been observed during this investigation, nevertheless it is the most obvious and likely explanation of these necrotic patches. The puncturing of the cells kills them, so that the granulation and condition of the walls may, therefore, be consequences of death. However, the granulation in the non-collapsed cells is not thus explicable.

Kostoff & Kendall (l.c.) talk of the " nutritive zone " in Cynipid galls, by which they mean that zone immediately round the focus of infection which is rich in nutritive substances, including proteins. They state that the presence of foreign irritants in the plant call for an abundant supply of nutriment, and that in the nutritive material, there are substances which react with the foreign ones. At the same time the increase of nutriment may cause molecular condensation and the final formation of proteins appearing in granular form. The same authors (1930) suggest that penetrating foreign substances induce antibodies which react with the foreign substances, causing precipitation. These ideas give a possible clue to the granulation in non-collapsed cells in *P. lanceolata* galls.

The term " nutritive zone " may be used for the granulated areas in the galls. The hypotheses of Kostoff & Kendall explaining the appearance of precipitations and protein granules are applicable, perhaps, to this " nutritive zone." Substances are secreted by the nematodes in the leaf of *P. lanceolata*, and there is a general reaction resulting in gall formation. At the places where the nematodes are actually in contact with cells, the concentration of the secretion is high, and the cell reaction is therefore greatest. At the focus of irritation there is a demand for increased nutritive material. Now having the increased amount of food substances various things may happen : the foreign substances may react with the protective substances in the food supply, resulting in precipitation. Or there may be molecular condensation forming proteins leading to protein granules. Further, the foreign substances may react with the excess of food substances, or induced antibodies, producing precipitation.

The difference to be seen in the starch grains in the gall, suggests that the starch is used up as a reaction to infection, for round the focus of infection the starch is either not present or is only very lightly stained by Iodine, whilst that further away is stained more deeply.

In Cynipid galls there is a zone of sclerenchyma surrounding the " nutritive zone," which Kostoff & Kendall (l.c.) consider to be " a product of plant reaction where the interaction between foreign substances and the plant protective substances takes place." They state, " It is a matter of fact that it (sclerification) is morphologically manifested as a reaction product in the plant tissue, where the plant substances inactivate the foreign substances into ones tolerable for the plant tissue." The formation of a zone of sclerenchyma tends to limit the gall. Thus the zoning of the gall is dependent upon the concentration of the gall-inducing substances.

When these ideas are applied to the galls on P. *lanceolata*, it is seen that the tissues change from the abnormal to the normal as the concentration of foreign substances diminishes with the distance of the tissue from the focus of infection. There is no zoning to be seen, the " nutritive zone " may be excepted, for it is the focus of infection. This change is explicable when it is realised that *P. lanceolata* shows no tendency to form sclerised tissue at all. The only tendency shown by *P. lanceolata* in the way of forming thickened tissue is seen in the presence of collenchyma. This occurs in the normal leaves, but in the galled ones there is more present, and this extra amount may be interpreted as a reaction to parasitization.

The formation of isolation areas by means of meristematic activity is of very common occurrence in wounded tissue. Quanjer (1927) in a paper on the eelworm disease of the potato plant, caused by *Anguillulina dipsaci*, found that necrotic patches in potato tubers tended to be isolated by meristematic activity of a cork cambial nature. The formation of isolated infection areas in the galls of *P. lanceolata* is of a similar nature to the process described by Quanjer ; though no trace of suberisation is to be found. There is an interesting point about the presence of these areas in *P. lanceolata*. In the galls collected in summer the areas of infection are not to be found, though hypertrophy and proliferation had taken place, but in fresh material collected in early January the traces of cambial activity only are found. Both are reactions of the same plant to the same stimulus, but since they are different it

must be the tone of the plant which is different at the two periods. Hypertrophy and proliferation occur when the tone is high, and wound cambial activity when the tone is low.

Thanks are due to Professor R. T. Leiper for granting facilities to collect material, and to Professor T. G. Hill for advice and suggestions in the writing of this paper.

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Three New Intermediary Vectors for *Syngamus trachea*

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OUR knowledge of the range of intermediate hosts of which *Syngamus trachea* can take advantage is increasing. Recent observations have shown that the centipede, *Scolopendra sp.*, the leatherjacket, *Tipula sp.*, and the spring tail, *Sminthurus viridis* may all act as vectors. In each case the larvae had migrated from the gut into the surrounding tissues where they had settled down. All these Arthropods have been found naturally infected on a plot of land known to be carrying a heavy burden of gapes.

Their importance in the transmission of gapeworm disease cannot yet be assessed, but *Sminthurus viridis* probably frequently acts as a transmitting agent to game birds, for it is known that young partridge chicks consume enormous numbers of this species in the first fortnight of their lives (Ford and others, 1938), though being so small they do not actually occupy very much space in the crop. They are extremely common and very active and therefore attract the attention of the young chick.

The leatherjackets, when infected, contained a number of Syngamus larvae, the range being 13–35. These developed to maturity when fed to chickens and produced typical cases of gapeworm disease. This host may possibly be considered as less important than the smaller springtails for it is a large larva and is unlikely to be tackled by a small chick. I have no information yet as to the effect of metamorphosis on the vitality of the gapeworm larvae, dormant within the tissues, but judging from recent results on the fly (Clapham, 1939), the larva can probably remain alive and unaffected by the changes. As the adult cranefly may often serve as food for birds, the leatherjacket may assume some importance as a reservoir of infection. Only 7 larvae have been examined and 5 contained gapeworm larvae, making a percentage of about 71.

The infected centipedes examined from this plot of land all contained multiple infections. Out of 16 specimens examined, 7 or nearly 44 per

cent. were infected and the highest number of larvae obtained from a single specimen was 17.

These percentages are high for natural infections and it may well be that over a wide area, similar examinations would give lower figures, for this focus of infection is localised on a small piece of land which in some way as yet unknown, came to carry gapes heavily. However, we must realise that these invertebrates mentioned above can act as filters and reservoirs of gapeworm infection, wherever they occur, and as such will assist in the maintenance and spread of the disease. They are all common insects, widely spread and in the aggregate may therefore be responsible for much loss to poultry and game farmers.

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On a Sex Difference in the Infection Rate of Birds with *Syngamus trachea*.

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IN 1937 a short paragraph by Whitlock appeared in the Journal of Parasitology commenting on the fact that there appeared to be a sexual difference in the degree of infection with *Syngamus trachea* of some Hungarian partridges which were being confined at the Michigan State Game Farm. Of 34 infected birds that he examined, 32 were females. He puts forward the suggestion that the strain of egg laying had lowered the vitality of the females to such an extent as to allow of the establishment of the parasite. We must assume therefore that all these birds were fully adult and that they were examined in the spring or early summer.

I have recently examined for helminth parasites close on a thousand partridges post-mortem and the data obtained throw fresh light on the question raised by Whitlock. These partridges were sent in from almost every English county and not from one estate only as were the American birds. Some were taken from the rearing field, others were shot or were found dead in the wild state and hence the group can be considered as a typical representation of English birds. There can be no reason to suppose that hen birds are exposed to infection more frequently than the cocks. The two sexes appeared in approximately equal numbers and the sample contained birds of every age—newly hatched chicks, poults, young adults and old birds. They were examined at all times of the year, being particularly abundant in the spring and summer, less so in the winter.

The age of the birds was assessed by the recognised method, from the shape and wear of the first primary feather and it was found that in partridges, as in most other birds, Syngamiasis is mainly a disease of youth. Infections were apparent in chicks from the age of about 10 days, poults were often affected, frequently heavily so. Adults were not immune for it has not been uncommon to find young adults harbouring an infection and indeed a number of birds in their second year also carried the parasites. Ten old males and one old female were infected but these numbers are too small to lead us to any definite conclusions as to the effect of increasing age on the susceptibility of birds to infection.

Out of 461 cock birds examined, 30 carried an infection and 9 of these had died of the disease. The number of females examined was 497, of which 72 were infected and 39 of them had died of Syngamiasis. Totalling these figures we have, 958 birds examined, 102 harboured the worms and 48 of these had succumbed to the infection.

The difference in the degree of infection between the two sexes from this sample of birds is very large, the ratio of the difference to its standard error being 4·57. When we consider the case mortality, the difference is less marked. Males seem to be less susceptible than females for the ratio is 2·22, a number which, however, is not high and too much notice should not be taken of it. However, if we consider the deaths due to *Syngamus trachea* in relation to the total number of birds examined, a different state of affairs seems to exist. The ratio of the difference to its standard error is 3·8, which is highly significant.

Other birds have been examined for the presence of *Syngamus trachea*, but the numbers are too small for statistical analysis. Yet experiments

on chickens carried out in this Institute tend to bear out the above conclusions. In all 36 chickens were used, 18 being cocks and 18 hens. They were infected with *Syngamus trachea* when 8 days old. A dose of 60 larvae was given to each bird. Two days later it was obvious that respiration was affected and the following day the first two chicks died. They were both females. Next day 3 more females were dead and the females continued to die for 3 more days, when all were dead. Two days later, *i.e.*, one week after the infections were made, the first male died. Within the next 5 days 12 males died. After a gap of a week another died. The others, though infected, survived the attack and recovered.

The numbers of pairs of worms recovered from these birds were :—

(*a*) from females—6, 7, 7, 8, 8, 8, 8, 9, 9, 12, 12, 14, 15, 15, 16, 19, 20, 21.

(*b*) from males—6, 7, 8, 9, 10, 10, 11, 11, 11, 12, 15, 16, 16, 21.

As regards intensity of infection there is little difference. The limits of 6–21 are identical but it is interesting to notice that with approximately equal infections, the females succumbed more quickly than the males. It is very striking that all the females died before the males even began.

These two observations certainly confirm Whitlock's conclusions that the hen bird is more susceptible to infection than the cock, but my experiments do not support his further conclusions that the strain of egg laying is an important factor. I am not able to put forward any reason for this difference.

Acknowledgment.

I am much indebted to Dr. B. G. Peters of this Institute for assisting me with the statistical analyses.

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The Physiological Ageing of Ancylostome Larvae.

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INTRODUCTION.

THE physiological age of infective larvae is of extreme importance in that it probably determines the infectivity of the larvae. Chronological age is not of such great importance, for larvae may exist under conditions which cause them to age rapidly in the physiological sense. Thus larvae at low temperatures become quiescent and do not use up their food reserves, remaining infective for much longer periods than do larvae living under environmental conditions which stimulate them to continuous activity. Hence in estimating the time infected areas will remain dangerous to man and to animals, the physiological age of the larvae concerned must be considered.

Payne (1922 and 1923), Cort (1925) and later Giovannola (1936) have shown that the physiological age of infective hookworm larvae can be determined by the examination of the fat stored in the larvae. The exact relationship between the fat content, activity and infectivity of the larvae does not, however, seem to have been clearly defined and the present work was undertaken to obtain, if possible, information to show whether the fall in fat content was directly correlated with the decrease in infectivity and activity. Accordingly larvae, some of which were aged rapidly and others which were aged slowly, were examined at regular intervals for glycogen and fat content, infectivity and activity.

PROCEDURE.

Larvae of *Ancylostoma caninum* (cat strain) were used in the investigation. Infective larvae were obtained by the usual faecal culture method. Two lots of larvae were selected and placed in jars containing water to a height of 2 mms. One jar was stored at 37°C. the other at 7°C.

Each week a number of larvae were taken from each lot and examined for (*a*) the presence of glycogen and fat, (*b*) infectivity and (*c*) activity.

(*a*) Staining methods were used for investigating the presence of fat and glycogen. Before fixation the larvae were allowed to stand in a 1/20 " Milton " solution in water. This caused the exsheathment of large numbers of larvae which greatly facilitated staining. As the larvae did not die during this period it seems unlikely that the fat or glycogen content was affected. Sharlach R. was used to demonstrate the presence of fat. In attempting to detect glycogen, Best's Carmine and Lugol's Iodine were utilised. For the examination of bodies of unknown origin, haematoxylin, methylene blue and methyl violet were used.

(*b*) Infectivity was estimated by means of the " floating raft " method (Goodey 1922 and 1925). The abdominal skin from a new-born mouse was stretched over a central hole in a flat cork. This " raft " was floated in physiological saline in a watch glass partly immersed in water at 37°C. Care was taken to ascertain that no air bubbles were imprisoned below the skin or cork. Larvae, usually 150 in number, contained in a small drop of water which usually evaporated in about 15 minutes, were placed over the hole in the cork and the apparatus allowed to stand for one hour after which the " raft " was removed. The larvae on the lower surface of the skin and cork were washed into the watch glass. Those on the upper surface were collected separately. The larvae in the two lots were then counted. Those on the upper surface of the skin were regarded as being uninfective, the others were considered to be infective. Calculation gave the percentage of infective larvae.

(*c*) Larvae taken from the two sources (one at 37°C., the other at 7°C.) were allowed to stand for one hour at room temperature after which larvae were placed on a slide on a warm stage at 30°C. When one minute had elapsed, the number of movements made per half minute by 20 larvae selected haphazardly were counted. The average number of movements made per second was calculated. This was considered to give an index of the activity of the larvae.

Observations.

1. *The Reduction in Fat Content.*—The young infective larvae were found to contain a large amount of fat, chiefly in the form of granules in or surrounding the intestine. Little fat was found in the oesophageal end though in young larvae a diffuse staining was observed in that region. As the worms aged, the size and number of the granules were reduced, until finally nothing remained but a very faint diffuse stain along the

line of the intestine. The diffuse fat in the intestinal cells was the last to be utilised by the worms.

Figure 1 shows the fat granules as they appeared in larvae stained at various intervals. As might be expected, the worms stored at 37°C. used up fat far quicker than the worms stored at 7°C. (see Table I). Evidently the high temperature stimulated the larvae to active movement causing a rapid reduction in the food reserves.

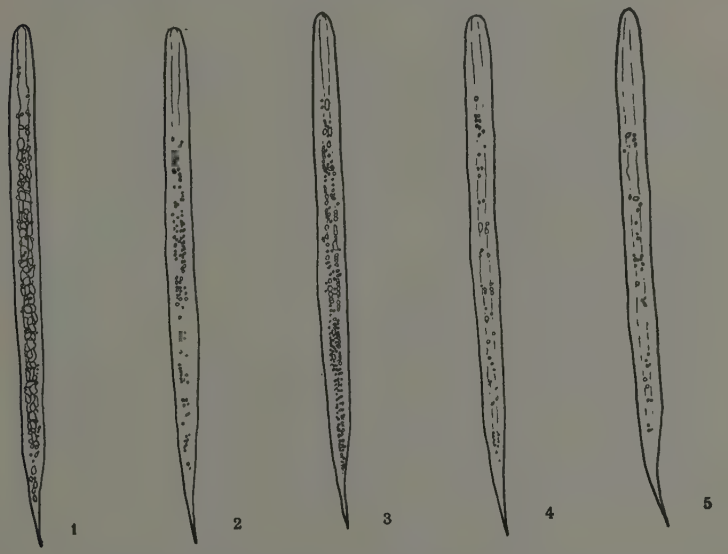


Fig. 1.—Granules staining with Sharlach R occurring in larvae of various physiological ages. For further explanation see Table I.

After three weeks the intestines of the larvae stored at 37°C. showed a number of very small dark granules which failed to stain with Sharlach R. As the larvae became older these particles increased greatly in number, extending from slightly behind the oesophagus almost to the anus. The particles did not appear to increase much in size, always appearing in small groups within the intestinal cells except in very old larvae, when isolated granules appeared in other tissues. In aged larvae the presence of the two lines of these dark granules, separated by the lumen of the intestine, was easily noted. The presence of the well-defined

cell X (probably the primordial sex cell, see Fig. 2) was a constant feature in ageing larvae. It was large and poorly defined at first, becoming smaller and more definite in outline as the larvae aged.

TABLE I.
FAT CONTENT.

Age.	Larvae at 37°C.	Larvae at 7°C.
Young larvae (immediately before storing).	Many large granules obscuring intestine. Oesophageal region fairly free. No. 1, Fig. 1.	
1 Week ...	Numerous small globules. Oesophageal and tail regions free. Diffuse stain throughout the larvae, especially in the intestine. No. 2, Fig. 1.	Few large granules. Very many small ones. Diffuse stain throughout the larvae, especially in the intestine. No. 3, Fig. 1.
2 Weeks ...	Stained granules almost absent. Diffuse stain confined to the intestine.	Few granules present, majority small. Intestine well defined by diffuse stain. No. 4, Fig. 1.
3 Weeks ...	Weak diffuse stain along intestine. Accumulation of small dark granules, non-staining, in intestinal cells.	Few fat granules; some diffuse stain in intestine. No. 5, Fig. 1.
4 Weeks ...	Very faint stain along the intestine. Dark granules, non-staining, increase. Lumen of intestine appears wide.	Few fat granules. Faint stain in intestine.
5 Weeks ...	Dark granules, non-staining, increase. Lumen of intestine appears wide.	Very faint stain in intestine.
6, 7, 8, 9, 10 and 11 Weeks.	The number of dark granules, non-staining, increase. Cell X becomes clearly defined.	Dark granules begin to accumulate. Cell X becomes clearly defined.

The nature of the dark granules found in the intestinal cells of old larvae is problematical. It seems likely that they arose as a result of the katabolic processes of the worm, the ageing of the larvae leading to an accumulation of excretory products. That these granules were not the same as those described by Payne (1922) and Giovannola (1936) was established by the disposition of the bodies, for they were not situated in the peripheral tissues of the larvae. Furthermore, Giovannola states that the granules he examined were of nuclear material, staining with Methyl violet and the Feulgen nuclear stains, whereas the granules found by the author failed to stain with Methyl violet, Methylene blue or Haematoxylin. In the assumption that these granules may have been excretory products of the nature of ketone bodies or lower fatty acids, attempts to obtain a colour reaction with sodium nitroprusside and sodium hydroxide, and with neutral ferric chloride, were made but

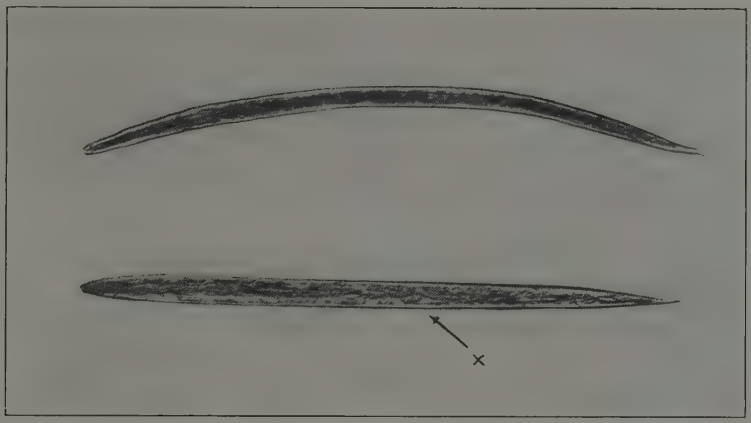
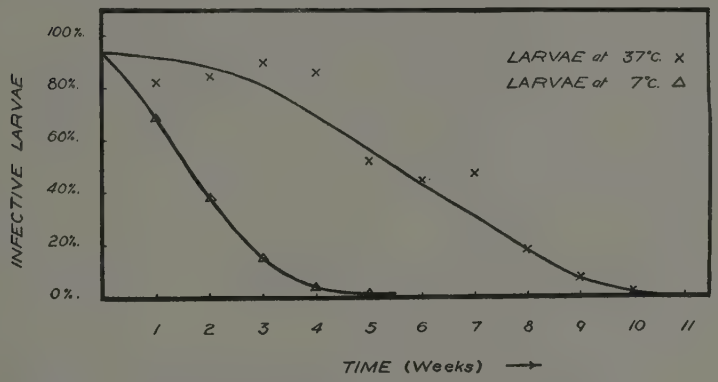


Fig. 2.—Photomicrographs of old (lower) and young (upper) 3rd stage larvae. The granules in the latter stained with Sharlach R, those in the old larvae failed to stain. For further explanation see text.

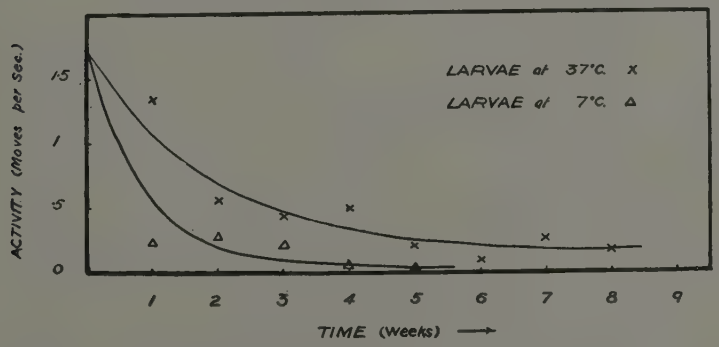
To face page 198.

proved unsuccessful. In all cases the larvae were exsheathed in 1/20 solution of " Milton " in water before staining was attempted. Gentle heat, just enough to kill the larvae, was found to disperse the granules.

At no time could the presence of glycogen be demonstrated by means of the staining techniques employed.



Graph I. The changes in infectivity in larvae stored at 37°C. and 7°C.



Graph II. The changes in activity in larvae stored at 37°C. and 7°C.

2. *The Changes in Infectivity.*—Graph I summarises the results of the infectivity tests carried out each week on rapidly and slowly ageing larvae. The importance of physiological age over chronological age is clearly illustrated. Payne (1923a) studied the relationship of infectivity to physiological age in larvae of *Ancylostoma caninum* but used " young "

and "old" larvae only, the former containing numerous granules, the latter very few. It does not appear that these larvae were stained. The young larvae were found to have a much higher infectivity than the old larvae.

3. *The Changes in Activity.*—Little need be stated in this connection for Graph II completely covers the results obtained. Using a very long and careful technique Payne (1923) investigated the activity of young and old hookworm larvae and found the former to be much more active than the latter. In view of possible practical applications the author's technique was as simple as was consistent with fairly reliable results.

4. *The Relation of Infectivity to Fat Content and Activity.*—Though Payne, as quoted previously, has examined the fat content, infectivity and activity in young and old larvae, it appears that no attempt has been made to correlate these factors throughout the life periods of ageing infective larvae. In the present investigation, the regular examination of larvae ageing at controlled rates from a condition of high infectivity to a condition of low infectivity, has enabled the relationship of these factors to be investigated further.

In the case of larvae stored at 37°C., the initial fall in fat content and activity was slightly greater than the fall in infectivity. Larvae stored at 7°C. showed this relationship in an even more marked manner. Thus at the end of the sixth week almost 50% of the larvae were infective, yet the fat content had fallen to a very small fraction of the initial quantity and the activity was only about $\frac{1}{8}$ of that of young larvae. In both cases a small percentage of the larvae remained infective when the fatty food reserves seemed exhausted. It appeared that there was a more definite relationship between the fat content and activity than between either of these and infectivity. Only in aged larvae did these three factors fall to a value of the same order.

Discussion.

The fact that infectivity fell more slowly than activity or fat content renders the estimation of the physiological age of larvae somewhat difficult. Furthermore, it seems probable that the rate of ageing may be important, the relationship being closer in rapidly ageing larvae.

The method of estimating the infectivity of the larvae may not have been satisfactory, for it seems likely that the proportion of larvae failing

to reach the host's intestine after successfully penetrating the dermis may have increased with the physiological age and this would give rise to a closer relationship between the three factors. If, however, the method used did give a true indication of the ability of the larvae to infect the host, it seems probable that the infectivity depends more on some unknown factor than on the food reserves or activity.

The close relationship between fat content and activity would be expected if it could be considered that the fat reserve indicated the amount of energy available for causing movement.

The elucidation of the nature of the dark granules accumulating, possibly as an excretory product, in the intestines of old larvae, may provide valuable assistance in the solving of the problems of nematode metabolism.

Summary.

1. The activity, infectivity and fat content of ageing infective larvae of *Ancylostoma caninum* were investigated at regular intervals.

2. The reduction in activity and fat content was found to occur at a similar rate.

3. As the larvae aged it was found that the initial fall in activity and fat content was greater than the fall in infectivity, especially in slowly aged larvae. Small numbers of larvae were found to be infective even when their fatty food reserves seemed exhausted.

4. Dark granules, possibly excretory products, were found to accumulate in the intestines of old larvae.

Acknowledgments.

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Helminth Parasites in Lambs on a Scottish Border Farm.

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ON farms in the Border Counties of Scotland, the sheep enterprise represents the chief line of production in both upland and lowland districts. Stocks of breeding ewes are run on practically all low-ground farms in the area, and these mainly consist of half-bred ewes (Border Leicester × Cheviot) which are mated with Oxford or Suffolk rams. The larger farms carry, in addition, their own flocks of Cheviot ewes for the production of half-bred ewe lambs for stock purposes. The number of cattle maintained on the pastures is comparatively small, though it is well recognised by farmers that an increase in their numbers would be to the advantage of the sheep.

The land is farmed on the semi-arable system in which the fields lie in grass for 6–8 years and are then ploughed up and put through a rotation of grain and roots for 3 years or thereby; but land near the farm steading is often worked on a more intensive rotation. There is therefore a constant renewal of the pastures as commonly practised in low-ground districts throughout Scotland.

The sheep stock is maintained almost entirely on grass except for about six weeks prior to lambing when ewes are folded on roots for a few hours each day but run back on to grass during the remainder of the day. On the heavier soils, the ewes are not folded but have roots laid down to them on grass fields. The pastures are, therefore, almost continually occupied by sheep, and owing to the heavy stocks carried, it is often difficult to rest the pastures to the extent desired.

The first year's ley is usually grazed by ewes and lambs and produces by far the best results owing to the relative cleanness of the ground and the high nutritive quality of the herbage. The rate of stocking is usually two ewes with double lambs, per acre, though it is occasionally much higher. In the case of older leys, 1½ ewes with double lambs, per acre, represents the average concentration. Second-year leys are reckoned to be about the worst for grazing purposes and are therefore hayed. The poor results are partly due to worm infection as a result of the heavy stocking in the previous year, and also to the small proportion of the finer herbage in the pasture, especially on the lighter soils.

Under the prevailing conditions, it will be realised that there is a high rate of infection with helminths. Accordingly, treatment of lambs and young sheep for these parasites is widely practised in the area, the ewe stock being frequently dealt with. Symptoms of worm infestation begin to appear about the beginning of June, and the most serious outbreaks occur in June and July. Trouble may be experienced, however, any time during the summer months until October. Affected lambs become unthrifty in appearance and fall back in condition, and there is usually diarrhoea of an intermittent type. Regular dosing with sulphate of copper alone, or in conjunction with nicotine sulphate, is carried out on many farms and is unquestionably of considerable value if begun early in the season. Experiments carried out by one of us (Corner, 1935) confirmed the value of this treatment in the area.

It was felt desirable to obtain full particulars of the occurrence of helminths on a typical farm in the area as there was little information on the subject apart from work carried out by Robertson (1937) who examined a few lambs from the district in 1933. Accordingly, it was arranged to take some 20 lambs from a representative flock in Berwickshire, and to make a complete record of all species of intestinal helminths found in them. The farm selected was situated in the Lauder district of Berwickshire and had previously experienced some loss from parasitic worms, though the extent of the trouble varied from season to season.

Conditions of Experiment.

In April, 1935, 32 half-bred gimmers with 64 twin lambs (Suffolk cross) which had been lambed at the end of March were put to graze on a 20 acre field, and as is customary in the district they were kept on the same field throughout the summer. The rate of stocking was 1·6 ewes

per acre, but, as grazing conditions were good during most of the experimental period, 12 half-bred ewe hoggs were grazed in the field, in addition, from 15th June to 30th July. All lambs were individually ear-marked and were weighed once monthly.

The field was situated at an elevation of 650 feet with a southerly exposure, and the soil was a medium loam on a somewhat open subsoil. The pasture was third year's ley in good condition, and had been grazed by sheep in each of the two preceding years. The animals received a balanced ration of concentrates at the rate of 1 lb. per ewe, but this was discontinued after 28th May. As the lambs were not run off, this was mostly consumed by the ewes. The health of the stock remained good throughout the summer, except for the occurrence of diarrhoea of an intermittent nature which was present in some of the lambs from the middle of June onwards. This was generally of a mild type, and though it gave the animals a temporary check, no marked symptoms of helminthic disease were observed up to the time of slaughter. No dosing or other treatment was administered. Mortality was restricted to one lamb which died in June from an unknown cause. The weather was very dry and cold during May, followed by considerable wet periods in June, but afterwards it became warm and genial. During the last fortnight of the experiment, however, the growth of the pasture was badly checked owing to drought and the lambs made little progress during this period.

On 19th August, 1935, 20 average lambs selected from the group were slaughtered and counts were then made of the intestinal parasites in each.

The average weights of these lambs at the beginning and end of the experimental period were as follows :

8th May.	19th August.	
Average weight.	Average weight.	Increase.
Lbs.	Lbs.	Lbs.
31·5	85·1	53·6

The average live-weight increase amounted to 3·6 lbs. per head, per week, so that notwithstanding the limited progress made by the lambs during the last fortnight, the average gain over the whole period was considerable. The lambs were in moderate store condition at slaughter.

Number and Species of Worms Recovered.

The results of worm counts are shown in the following table.

No. of Lamb.	*Haemonchus contortus*	*Ostertagia circumcincta*	*Ostertagia trifurcata*	*Trichostrongylus axei*	*Trichostrongylus vitrinus*	*Trichostrongylus colubriformis*	*Nematodirus filicollis*	*Cooperia curticei*	*Strongyloides papillosus*	*Trichuris ovis*	*Chabertia ovina*	Totals.
1	594	4,184	1,404	—	108	—	119	119	10	37	135	6,710
2	77	1,873	456	183	88	29	354	88	88	57	197	3,490
3	4	706	164	4	132	—	1,175	267	66	14	79	2,611
4	158	3,641	578	241	240	48	96	96	960	31	150	6,239
5	81	799	326	43	25	—	1,229	—	12	90	150	2,755
6	2	1,382	350	—	72	—	288	254	234	46	135	2,763
7	109	596	128	28	141	—	6,004	283	106	24	138	7,557
8	640	11,321	2,220	1,110	246	—	14,700	—	123	40	77	30,477
9	33	2,906	341	85	112	—	675	90	—	23	53	4,318
10	268	3,903	681	250	—	—	2,824	651	431	48	172	9,228
11	2	1,622	549	128	317	—	3,806	238	—	40	70	6,772
12	111	4,574	654	952	288	—	976	632	404	54	32	8,677
13	125	2,456	729	56	50	—	3,108	—	50	24	105	6,703
14	129	8,317	2,959	—	130	—	6,124	260	65	51	91	18,126

No. of Lamb.	*Haemonchus contortus*	*Ostertagia circumcincta*	*Ostertagia trifurcata*	*Trichostrongylus axei*	*Trichostrongylus vitrinus*	*Trichostrongylus colubriformis*	*Nematodirus filicollis*	*Cooperia curticei*	*Strongyloides papillosus*	*Trichuris ovis*	*Chabertia ovina*	Totals.
15	—	168	67	89	146	—	122	72	96	65	173	998
16	440	1,824	314	26	97	—	3,896	291	339	111	272	7,610
17	12	1,078	242	188	136	27	787	81	40	74	164	2,829
18	15	4,367	723	303	324	49	712	97	16	61	83	6,750
19	15	472	136	157	52	—	52	—	40	32	116	1,072
20	40	765	118	17	72	—	6,452	218	109	4	114	7,909
Totals	2,855	55,494	13,139	3,860	2,776	153	53,499	3,737	3,189	926	2,506	143,594
Average	142·8	2,774·7	657·0	193·0	138·8	7·7	2,675·0	186·9	159·5	46·3	125·3	7,179·7

The figures given in the above table show that a considerable proportion of the lambs examined harboured a fairly heavy worm population and that the infestations varied greatly in spite of the fact that the animals had been grazed on the same pasture and were approximately of the same age. The total counts range from below 1,000 worms in lamb No. 15 to over 30,000 in lamb No. 8, although over half the lambs give a figure around the general average of about 7,000 worms.

Ostertagia circumcincta was the most common of all the species found, and in two of the lambs over 8,000 worms were counted, a figure which is thought to be necessary before disease symptoms are produced. *O. trifurcata* was also found in all the lambs although the numbers, as is generally the case with this species, were considerably lower.

It is generally considered that *O. circumcincta* is not only the most common, but also the most frequent cause of loss, of all the intestinal worms in Scottish sheep, and that *Haemonchus contortus* is, as a rule, of little importance. The figures given above for the latter species show, however, that it is by no means negligible and that it is common enough to cause serious losses when conditions for its development are favourable. Since treatment against stomach worms in sheep is now widely practised, it is probable that outbreaks of parasitic gastritis resulting from heavy infestations with *H. contortus* occur less frequently than they do from *O. circumcincta* as the former is more readily expelled by the anthelmintics in common use. Two of the lambs examined in the present investigation gave counts of over 500 for *H. contortus*, and this figure represents a fairly heavy infestation for this species.

From the point of view of numbers, *Nematodirus filicollis* follows *O. circumcincta* very closely as one of the commonest intestinal worms in lambs. In one of the lambs 14,700 individuals were found and this represents the highest figure for any one species in the investigation. Three other lambs gave counts of over 6,000 worms for this species. The average number per lamb is only very slightly below that obtained for *O. circumcincta*.

Evidence of the pathogenicity of *Nematodirus spp.* in sheep is conflicting and although they are frequently the most numerous forms in the small intestine, the tendency is to regard them as of less importance than species of the genus *Trichostrongylus*. Tetley (1935) found no outward signs of infection in lambs harbouring 5,000 individuals, and Kausal (1937) found no appreciable effect on the body weight of a sheep carrying

an experimental infection of 6,300 worms. In fact the latter author (1933) records up to 32,000 *Nematodirus spp.* in a fat lamb. Gordon (1936), on the other hand, noted some loss of condition and diarrhoea in sheep where these species formed the greater part of the infection. In the north-western states of America it is recorded (Anon., 1938) that *Nematodirus* has the most severe pathogenic effect of all intestinal parasites in sheep.

When heavy infestations with *N. filicollis* are found in lambs in this country other species are usually present in such numbers that it is difficult to form a definite opinion on its pathogenicity, and although the pink colour of the worms strongly suggests a blood-sucking habit it seems probable that large numbers would be required before disease symptoms become evident.

The other species obtained in this series do not call for special comment except for the low incidence of *Trichostrongylus colubriformis* and the fairly high incidence of *Strongyloides papillosus*. The latter species was found in all but two of the lambs although the numbers were relatively small.

Other intestinal forms not recorded in the above table were *Moniezia expansa* in light infections in three of the lambs, and *Capillaria longipes* in one, also a light infection.

The more general survey of helminths in sheep in Scotland carried out by Robertson (1937) gave much the same general picture of the worm infestation as that obtained in the present investigation. The somewhat larger proportion of heavy infestations which Robertson found may be accounted for by the fact that a large number of the lambs he examined were showing advanced symptoms of helminthic disease.

Even when no outward symptoms of disease are evident, it would appear from the present investigation that, on a typical Border farm in Scotland, a considerable worm population occurs in a large proportion of the lambs and that the pastures are heavily contaminated with infective material. The heavy stocking of the land, as is the usual practice in the district, ensures a rapid increase in the number of infective larvae on the pastures when conditions for their development are favourable, and were it not for the extra feeding with concentrates and anthelmintic treatment which are now becoming more widely practised, it is probable that serious outbreaks of parasitic gastritis would occur far more frequently.

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Studies on the Saline Requirements of the Larvae of *Ascaris suum.*

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INTRODUCTION.

UNTIL comparatively recently, the greater part of research work in helminthology progressed along three main lines, investigation into the life histories of parasitic helminths, the study of their morphology as well as an empirical investigation into the efficiency or otherwise of different substances as anthelmintics. Research into the physiology of these animals was only rarely attempted and even then was only very casual and never by any means thorough. In recent years, however, there has been a gradual change over, and increased attention has been paid to their physiology, both from a purely scientific and also an applied standpoint. Early work on anthelmintics was concerned only with the discovery of substances which would expel the worms from their hosts and could be used empirically. This attitude has of recent years, given place to a more scientific approach in which investigations into physiological processes have been undertaken in the hope that knowledge gained might show some method of rendering the worms more susceptible to anthelmintics, or alternatively, indicate new anthelmintics more lethal to them and less harmful to the hosts.

Research into the physiology of parasitic worms is however, by no means easy. Inside the hosts, they are very inaccessible and experiments on them are almost impossible. The fact that the worms are obligatory parasites makes it impossible to study their physiology outside the hosts, since once removed from it they are in an unnatural habitat which in all probability affects their physiological processes; one is therefore no longer working on a normal healthy animal but on an organism in a pathological condition. In order that results obtained should have any value, it is important that the work should be carried out on a normal

healthy specimen, since it is obvious that in such an animal only would metabolic processes occur in a normal manner. The need for maintaining the worms in a healthy condition thus becomes apparent and an essential preliminary to any accurate or thorough investigation into their physiology is the evolution of a culture medium in which the worms can live in a healthy condition.

It would be interesting to consider at this point what are the essentials of a true culture medium. In the first place, its constitution should be such that its chemical and physical properties are not in any way harmful to the worms. They should in fact, approximate as closely as possible to conditions encountered in the host. In the second place, sufficient nourishment should be present for the worms to develop or if mature, to reproduce. The eggs produced in culture should be perfectly normal and capable of carrying on the normal life cycle. When a medium fulfils these conditions it is very probable that it presents a normal habitat to the organisms in it, and one is justified in considering the latter as being normal and healthy.

In planning a series of experiments on the culture of helminths, two methods of procedure present themselves. One is to start with the adult worm recovered from the host and maintain it in a healthy condition ; another is to start with infective eggs or larvae and induce them to develop to the adult form. At first sight the former appears the more promising method, but consideration shows that certain factors make it very difficult. In the first place one cannot be certain that the worms obtained are uniform either in age or physiological state. An additional difficulty is experienced in the culture of these worms as it is almost impossible to render them aseptic. If a nutritive medium is used, growth of bacteria present will be very rapid and will very soon change the composition of the medium and render it unsuitable for the worms. If on the other hand, one adopts the latter procedure, then the difficulties attendant on sterility are not insuperable, since it is comparatively easy if one chooses a species whose infective stage is an infective egg containing a larva to sterilise the surface of the egg. The problem then resolves itself to finding a method of hatching the egg and cultivating the larva liberated. It is also probable that the young larval stages produced are less specialised in their physiology than are adults and consequently results obtained from working on the larvae of one species would be reasonably true for other species.

For the reasons mentioned, it was decided that the larvae of *Ascaris suum* were the most suitable for research. This worm is easy to obtain in large quantities from the intestines of slaughtered pigs. The eggs can be recovered from the adult female in great numbers and the incubation of these results in a very large number becoming infective (95–99%). A previous paper by the author deals with the *in vitro* hatching of these eggs (Fenwick 1939).

It has already been suggested that the first essential of a true culture medium should be that its chemical and physical conditions are not in any way harmful to the larvae and it was accordingly decided that the immediate problem to be solved was the evolution of a saline solution properly balanced, in which the larvae could exist in the most natural condition. This has been done in very great detail for vertebrate tissues, but as far as parasitic worms are concerned, little or nothing in the way of systematic research has been accomplished apart from the work of Lapage (1935). The latter confined himself to haphazardly determining the effect of a number of standard salines on the fourth stage larvae of Trichostrongylids, ultimately deciding that best results were obtained using Peter's medium. It was felt by the author that experiments on the effect of different salines did not go deeply enough into the problem, and the conclusion was reached that the only satisfactory method of reaching a real solution to the problem was to make detailed observations of the effect on the worms of varying concentrations of inorganic salts in the presence of one another. It was felt that this would result in the direct evolution of a saline, suitable for the larvae.

It would be interesting to consider at this stage, the work carried out by different workers on the effect of varying concentrations of inorganic salts on animal tissues. This work is admirably reviewed by Willmur (1928) from whose paper references may be obtained. It was found that although the number of papers published was large, the majority were merely records of a number of disconnected experiments in which the effect on the tissues of different combinations of sodium, potassium, calcium and magnesium chlorides, chosen presumably at random, was determined. The most interesting work appears to have been carried out by Willmur (1911) and by Lewis & Lewis (1911).

The latter conducted a very critical and detailed series of experiments on the tissues taken from embryo chicks varying the constituents of the saline both independently and also in combination with variations in

other constituents. The interpretation of his results, however, was complicated by the fact that different media were most suitable for different results, *e.g.*, one was most active in promoting migration, but life of the tissue in it was short, another was relatively inactive in promoting migration but the survival time of the tissue in it was longer.

Willmur's work was on the same lines as that of Lewis & Lewis, but glucose was included in his solutions. He stressed its importance as an agent responsible for stimulating the tissues to active migration, giving its optimum concentration as 1%, above which it appeared to have a toxic effect. It is doubtful from the results given, whether or not its presence affected the length of life of the tissues.

It was felt that in the evolution of a saline the first attention should be paid to the proportions of the metallic ions present. The question of bicarbonate content and glucose content could safely be left until this had been attended to, as in all probability bicarbonate content was only important in so far as it affected the *p*H of the medium, while the glucose on the other hand was probably related to the energy sources of the medium ; in other words, the action of one was physical while the other was that of a food. This assumption that bicarbonate content was unimportant except in so far as it affected the *p*H of the medium is supported by Davey (1938). As a result of a series of experiments on *Ostertagia circumcincta* he came to the conclusion that the inclusion in the medium of disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium bicarbonate and magnesium chloride did not result in any increase in the life of these worms. He evolved a saline consisting of 0·9% NaCl, ·042% Kl and ·024% $CaCl_2$, which he claims to be suitable as a basis for media for the cultivation of *Ostertagia*.

The experiments to be described in this paper are therefore concerned firstly, with the determination of the optimum concentration of metallic ions in order to ensure the highest degree of ionic antagonism as far as the larvae of *Ascaris suum* are concerned, secondly, to investigate the tolerance of the larvae to variations in *p*H and lastly to determine the effect (if any) on the larvae of the addition of glucose and other carbohydrates to the saline.

Technique.

In the experiments described below, infective eggs were obtained by incubating eggs obtained from the uteri of adult female worms in a

shallow layer of water in petri dishes at 30°C. Bacterial growth was reduced to a negligible extent by changing the water in the dishes on alternate days, each time washing the eggs with several changes of water. Brown (1922) used as a medium a 1 : 1,000 dilution of formalin. The use of this medium was discontinued for two reasons : in the first place it was found that the formalin tended to encourage the growth of yeasts and moulds, secondly it was found that formalin-cultured eggs subsequently proved remarkably difficult to hatch. Under these conditions, the eggs developed rapidly and uniformly, becoming embryonated in 10–14 days. They were not regarded as infective and ready for hatching until they had been incubated for a further 14 days, since Stoll (1933) proved that embryonation of a nematode egg is not in itself evidence of infectivity.

Hatching of the infective eggs was accomplished by treating them with a 1 : 15 dilution of "Milton" for twelve hours at 38°C. Subsequent pressure of the eggs between a slide and coverslip caused the ejection of the larvae in a living condition. They were then washed six times with the experimental saline and were then ready for culturing. Great care was taken not to allow the temperature of the larvae to fall far below 38°C. for any length of time after hatching, as it was found that the hatched larvae were very sensitive to a fall in temperature, ten minutes at room temperature being sufficient to kill 90–95% of those present. In order to minimise this danger, all solutions were stored in an incubator at 38°C. until immediately before use, and all operations were conducted as rapidly as possible. When the technique was correctly observed, it was found that a culture containing 85–90% living larvae could be obtained.

Three methods were employed for culturing the larvae. The commonest was to use solid watch-glasses, containing the larvae in the experimental medium and sealed with glass plates smeared with vaseline. A number of cultures were made up in Carrel flasks approximately one inch in diameter and a quarter of an inch in depth. In a few cases hanging drop cultures were utilised. All three methods proved satisfactory and gave comparable results, the first two being generally utilised as they were far easier to set up than was the hanging drop technique. Generally speaking no attempt was made to secure asepsis as the solutions used were non-nutritive and no bacterial growth was experienced in them. In a few cases aseptic Carrel flask cultures were set up but the life of the

larvae in these was not significantly different from that of control cultures made up without aseptic precautions.

Each experiment consisted of observing the life span of the larvae in a series of salines differing from one another in the proportion of one component present; each member of the series was represented by six individual cultures and the life span in that member was taken as being the average of the six. Each experiment was repeated six times. Graphs were drawn for each series in which the percentage of the variable constituent was plotted against the life span of the larvae. Each experiment being repeated six times, six graphs were obtained for each series; these were subsequently superimposed on one another. In this way a fairly accurate idea of the optimum concentration of each constituent was obtained.

In the case of watch-glass and Carrel flask cultures there were usually present about 2,000 larvae, while in the hanging drop preparations approximately 300–400 larvae were involved.

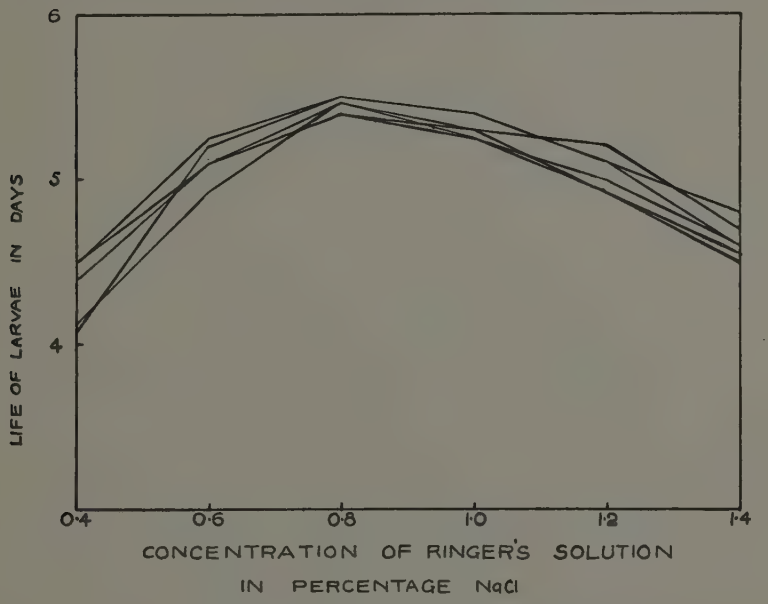
It is interesting to consider at this stage, the behaviour of the larvae in salines generally. It was found, unless a particular saline was completely unsuitable, that the larvae on being introduced into it lived for a period in an active and apparently healthy condition. This period was followed by a second when the movements were considerably slower; this second period was very short and terminated in the death of approximately 80–90% of the larvae present. The remainder lived for a varying time showing only feeble movement. It is considered that the first period represented the time of healthy survival of the larvae in the saline, the second and succeeding stages representing the onset of moribund or pathological conditions in the larvae. Individual variation would account on the one hand for a few larvae which lost their activity early, and on the other for a small number which remained active for longer periods than did the rest of the larvae in culture. As it was intended that these experiments should be concerned with healthy larvae in a normal condition, the first period was taken as representative of the life span of the larvae in salines. An advantage resulting from adopting this as a standard lay in the fact that although there were frequently considerable variations in the time elapsing before death supervened in different cultures made up in any one saline, the period already mentioned showed a far greater degree of uniformity than did any other. In practice no difficulty was experienced in recognising the onset of the moribund

condition. For these reasons this is the period referred to in all results given for this series of experiments.

All experiments were conducted at 37·5°C.

Procedure.

1. *Total Ionic Concentration and its Relation to Longevity of the Larvae.* —It was considered that a most important preliminary to determining the optimum ionic concentration of each salt present in the saline was an



Graph 1. Effect of Different Osmotic Pressures on the larvae.

investigation into the relationship between the total ionic concentration of a saline and the longevity of the larvae. Data obtained from such an investigation would indicate the optimum value for the total ionic concentration, in other words would give a measure of the most suitable osmotic pressure of the medium. In view of the fact that the larvae spend a part of their life in the blood, it was not considered likely that ordinary mammalian Ringer's solution would contain any substance toxic to the worms in the concentration normally encountered in this

solution ; it was moreover, felt that the ionic antagonism in this saline was in all probability, fairly close to that required by the larvae.

A standard Ringer's solution containing 0·8% NaCl, 0·02% KCl, 0·02%$CaCl_2$, and 0·01% $MgCl_2$ was accordingly made up double strength, and diluted to form a series containing 1·6%, 1·4%, 1·2%, 1·0%, 0·8%, 0·6% and 0·4% NaCl, with corresponding concentrations of other salts. The life of the larvae in these was determined. It will be seen that these solutions are deficient in bicarbonate and in glucose, the reason for this absence being mentioned earlier.

The results of this experiment are set out graphically in Graph 1. It will be noted that the larvae are not very sensitive to small changes in concentration around the optimum value, which as can be seen, is that of a Ringer's solution containing 0·8% NaCl and corresponding proportions of other salts. This concentration can be dropped to 0·7% or raised almost to 1·1% without any serious shortening of the life of the larvae.

It is interesting to note the rapid shortening of life as the concentration is dropped below 0·7% and to contrast this with the very gradual shortening as the concentration is raised above 1·1%. This can be correlated with the behaviour of the larvae in the two kinds of medium. In concentrations lower than 0·7% NaCl, the larvae were exceedingly active but their movements were limited in scope. Their movements were exceedingly rapid—more rapid than those of larvae in the optimum concentration, but were not very great in extent. This phenomenon might be due to the fact that the organisms were hypertonic to the medium, the resulting increased turgor of their bodies effectively preventing them from performing the agile movements characteristic of larvae in more concentrated solutions. The onset of the moribund condition in dilute salines was sharp and well defined.

In the more concentrated solutions a different state of affairs existed. In these, the movements of the larvae were slow but very great in scope. This might be due to the decreased turgor of their bodies resulting from their being hypotonic to the medium, causing them to become excessively flexible, but at the same time causing them to lose the power of rapid movement. The onset of the moribund condition in the more concentrated media was usually gradual and ill-defined.

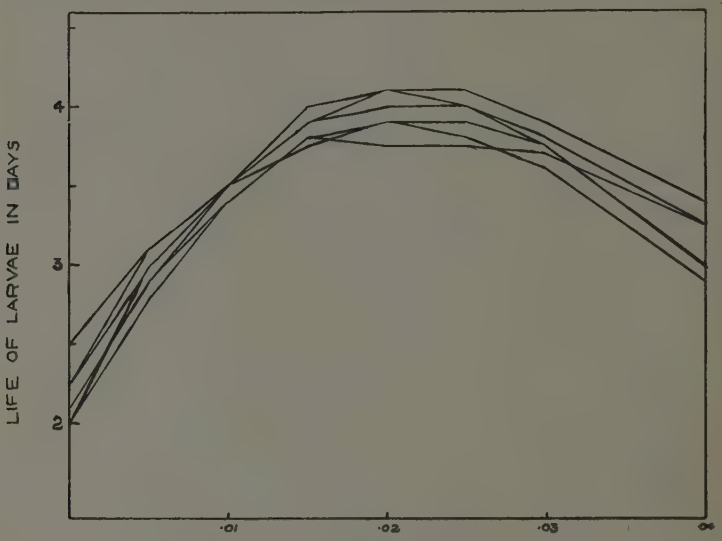
In view of the results obtained it was decided that the optimum osmotic pressure of a culture medium for the larvae was that of a Ringer's

solution containing 0·8% sodium chloride and corresponding concentrations of the other salts. Calculation will show that this corresponds in total ionic concentration to that of a 0·832% simple saline solution, and it was therefore concluded that as far as total ionic concentration (in other words osmotic pressure) was concerned a simple unbalanced saline containing 0·832%NaCl would be perfectly satisfactory as the basis for culture media.

It is important however, not to overlook the fact that such a simple saline as already mentioned would not be at all suitable as the basis of a medium owing to the presence in it of an unopposed sodium ion which would have a toxic effect on the organisms. It was accordingly decided that the next step in the investigation must be the determination of the ionic concentration of other ions like potassium, calcium and magnesium, necessary in order to secure the highest degree of antagonism to this toxic effect.

2. *The Effect of Addition of Potassium Chloride to the Simple Saline.*— The effect of the addition of potassium chloride was first determined. This might be accomplished by addition to the 0·832% NaCl solution, varying quantities of the salt. If this were done without altering the concentration of the sodium chloride, then the osmotic pressure of the mixture would be raised and it was considered that although the larvae are remarkably insensitive to small changes in total ionic concentration, it was better to eliminate this factor completely. For each addition of potassium chloride therefore, the concentration of the sodium chloride was dropped by a fixed amount. Consider a 0·832% simple saline solution ; the molecular weight of sodium chloride being 58·5, its molar concentration would be 0·143 moles per litre. The molar concentration of potassium chloride in a solution containing ·005% of this salt would be ·00067 moles per litre. These two salts in solution are completely ionised and hence the osmotic pressure exerted by each is proportional to its molar concentration. Consequently it may be said that the total of the molar concentrations of individual salts gives a measure of the total osmotic pressure of the solution or, in other words, the total ionic concentration. If this is kept constant then the osmotic pressure of the solution will be independent of the differences in the ratio of its constituents. The question therefore resolves itself to merely maintaining the total molar concentration of salts in solution at a constant

value, in other words for the addition of ·005% KCl, the concentration of NaCl must be dropped by ·00392%. Higher concentrations of potassium chloride would involve correspondingly greater decreases in the concentrations of the sodium chloride. As a result of calculations on these



Graph 2. Effect of Addition of KCl to saline.

lines the following series of solutions was made up and the life of the larvae in them determined.

0·832% NaCl	... ...	0% KCl
0·828% NaCl	... ...	·005% KCl
0·824% NaCl	... ...	·010% KCl
0·820% NaCl	... ...	·015% KCl
0·816% NaCl	... ...	·020% KCl
0·812% NaCl	... ...	·025% KCl
0·808% NaCl	... ...	·030% KCl
0·800% NaCl	... ...	·040% KCl

As in experiments on the total ionic concentration, six experiments, each comprising six individual cultures, were performed, the results

being set out graphically in Graph 2. Reference to this graph discloses the fact that in a simple saline solution the larvae are only able to live for 2–2½ days. Addition of potassium chloride to the saline results in a lengthening of the life of the larvae up to a maximum of 3¾ to 4 days in a saline containing 0·20% of this salt. Decrease in the concentration of the potassium chloride to 0·15% or increase to 0·025% did not appear to have any appreciable effect on the larvae, but a variation of the concentration outside these limits resulted in a shortening in the life of the larvae. 0·020% was therefore considered to be the optimum concentration of potassium chloride for the larvae.

3. *The Addition of Calcium Chloride and its Effect on the Larvae.*— This series of experiments were carried out in a similar manner to those on potassium chloride. A simple saline solution containing 0·02% potassium chloride was taken and varying quantities of calcium chloride added to it. As in the previous series the concentration of sodium chloride was dropped for each addition of calcium chloride, the resulting solutions being as follows :—

0·816% NaCl, 0·020% KCl, 0% $CaCl_2$.
0·813% NaCl, 0·020% KCl, 0·005% $CaCl_2$.
0·811% NaCl, 0·020% KCl, 0·010% $CaCl_2$.
0·808% NaCl, 0·020% KCl, 0·015% $CaCl_2$.
0·806% NaCl, 0·020% KCl, 0.020% $CaCl_2$.
0·803% NaCl, 0.020% KCl, 0·025% $CaCl_2$.
0·800% NaCl, 0·020% KCl, 0·030% $CaCl_2$.
0·795% NaCl, 0·020% KCl, 0·040% $CaCl_2$.

The results of this series of experiments are set out in Graph 3. It will be seen that the life of the larvae is increased to approximately 4¾ days when the concentration of calcium chloride is raised to 0·20%. There is a marked insensitivity on the part of the larvae to higher concentrations of calcium chloride, even at 0·40% the life of the larvae averaged out to 4½ days. The results obtained for the effect on the larvae of calcium, are in marked contrast with those of Davey (1938) working on *Ostertagia circumcincta*. He found that calcium did not appear to exert any antagonistic effect on the sodium and merely included it in his solution since he considered it " improbable that the worms could live their normal life span without calcium." He found that the addition of 0·24% calcium chloride to a simple unbalanced saline solution merely delayed the onset of death, the maximum survival period being three days,

whereas in ordinary saline it was one day. In experiments of this kind, however, delay in the onset of death is the only criterion by which the suitability or otherwise of any constituent can be evaluated, since one is dealing with non-nutritive solutions and therefore, in fact, measuring the starvation survival period under different conditions of starvation. Consequently, any increase in the survival time as a result of the addition of any new constituent must be regarded as indicating a beneficial effect exerted by that constituent, and the increase in survival time as a result of the addition of calcium chloride (one day to three days, *i.e.*, a 200% increase), must indicate an inhibitory effect exerted by the calcium on the sodium.

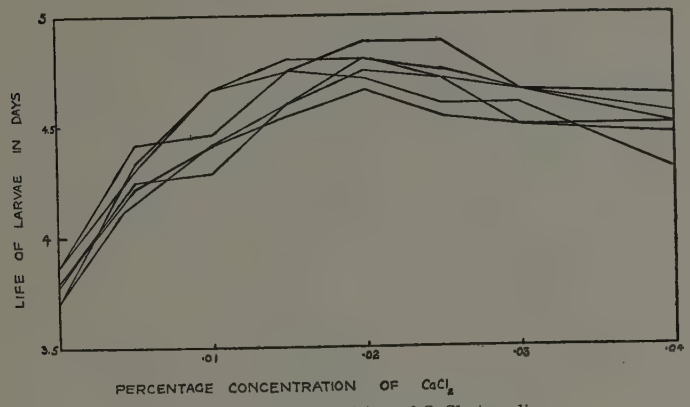
In view of the fact that the maximum beneficial effect was exerted at a concentration of 0·02% $CaCl_2$, this was taken as the optimum concentration of this salt and all future salines were made up containing this concentration.

4. *The Addition of Magnesium Chloride and its Effect on the Larvae.*— The technique in this series of experiments was identical with the technique followed in the previous experiments. The solutions made up were of the following constitution :—

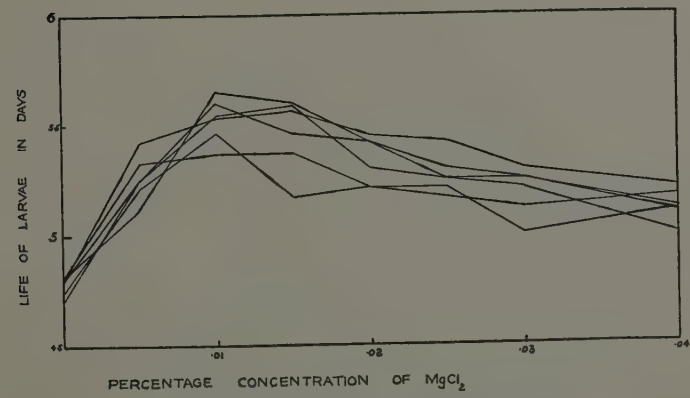
0·806% NaCl, 0·020% KCl, 0·020% $CaCl_2$, 0% $MgCl_2$.
0·803% NaCl, 0·020% KCl, 0·020% $CaCl_2$, ·005% $MgCl_2$.
0·800% NaCl, 0·020% KCl, 0·020% $CaCl_2$, ·010% $MgCl_2$.
0·797% NaCl, 0·020% KCl, 0·020% $CaCl_2$, ·015% $MgCl_2$.
0·794% NaCl, 0·020% KCl, 0·020% $CaCl_2$, ·020% $MgCl_2$.
0·791% NaCl, 0·020% KCl, 0·020% $CaCl_2$, ·025% $MgCl_2$.
0·788% NaCl, 0·020% KCl, 0·020% $CaCl_2$, ·030% $MgCl_2$.
0·732% NaCl, 0·020% KCl, 0·020% $CaCl_2$, ·040% $MgCl_2$.

The results are set out in Graph 4 and it will be seen that the addition of magnesium chloride increases the life of the larvae, the optimum effect being obtained with a concentration of 0·010% of this salt, when the life of the larvae is increased from $4\frac{3}{4}$ days to $5\frac{1}{2}$ days. An increase of concentration of magnesium beyond this results in a gradual falling off in the period of healthy life. It thus appears from the experiments described in this and in preceding sections that the most suitable saline solution for the larvae as far as total ionic concentration and ionic antagonism are concerned, is of the following constitution :—0·80%NaCl, 0·020% KCl, 0·020% $CaCl_2$, 0·010% $MgCl_2$, the life of the larvae in this solution being approximately $5\frac{1}{2}$ days.

5. *Variations in pH and their Effect on the Larvae.*—Having evolved a saline correctly antagonised for the larvae, it was considered advisable



Graph 3. Effect of Addition of $CaCl_2$ to saline.



Graph 4. Eflect of Addition of $MgCl_2$ to saline.

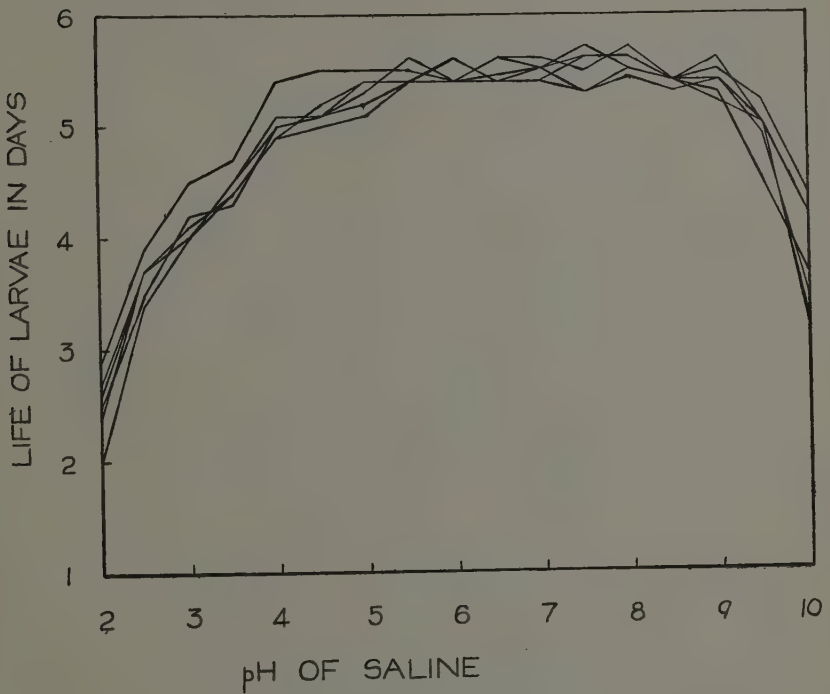
to adjust its *p*H to the most suitable value for the continued life of the larvae. Previous work indicates a marked insensitivity on the part of parasitic nematodes to *p*H fluctuations. Lapage (1935) used media ranging from *p*H 3·6 to *p*H 9·6 and found that the third stage larvae of

Trichostrongylids were remarkably tolerant to variations above *p*H 5·0 ; below this figure the tolerance did not extend to any great extent. Davey (1939) showed that the abomasal worms of the sheep were able to tolerate variations from *p*H 4·0 to *p*H 9·0. Both these results, however, were obtained with species inhabiting the intestine, which in all probability presents a more variable environment than does the blood stream, the habitat of the *Ascaris* larvae. It was accordingly felt that it would not be at all surprising if the larvae were found to be more sensitive to *p*H variations than were the worms already mentioned.

A preliminary series of experiments using balanced salines buffered to values between *p*H 5·6 and *p*H 8·0 showed that the larvae were not in the least sensitive to variations between these limits. A series of experiments was also conducted with the object of determining the approximate limits in *p*H values which the larvae could tolerate. In order to eliminate any possible toxic effect due to the presence of buffering substances, it was decided to dispense with the use of buffered solutions, and to rely on the addition of acid and alkali to control *p*H. A series of solutions was accordingly made up ranging from *p*H 2·0 to *p*H 10·0. In order to minimise the effect of any changes in *p*H as a result of the discharge of excretory products by the larvae, the cultures were made up in Carrel flasks and the medium was changed every twelve hours. *p*H determinations made on the old medium after removal indicated that under these conditions, any fluctuations which occurred were too small to be detected. All determinations were conducted in a disc colorimeter, the readings being considered to be accurate at least to $\pm$0·1.

Six experiments were performed, the results being illustrated in Graph 5. Reference to this graph shows that the larvae can tolerate a *p*H range of approximately 5·0 to 9·0 without any serious shortening of life. In practice it was found that individual cultures often tolerated far wider ranges even than this. It should be borne in mind, however, that the figures given are not by any means constant for individual cultures. Different cultures varied considerably in their tolerance to *p*H variations. It is therefore not practicable to lay down any hard and fast rules as to the limits of tolerance of the larvae. It is also necessary to bear in mind the fact that the tolerance of the larvae to *p*H variations under conditions of starvation might be different from their tolerance under normal conditions. It is by no means inconceivable that the physiology of nutrition of the worms might be such, that it can only

proceed normally within certain narrow limits. Information on this point can never be obtained with any certainty until more is known regarding the physiology of the worms and the nature of their food. The conclusion was therefore reached that unless the *p*H of the medium fell outside the limits of 5·0 and 9·0 it could be disregarded.



Graph 5. Effect of *p*H on the larvae.

As the *p*H of the medium did not appear to have any effect on the larvae, it was considered improbable that the inclusion of sodium bicarbonate in the medium would affect the larvae. Experiments, however, were conducted in which the effect of addition of this substance to the medium was determined. These were conducted in the same way as were previous experiments and due allowance for increase in total ionic concentration as a result of the addition of bicarbonate, was made

as before. The results were fully in accordance with expectations, the added bicarbonate having no apparent effect on the larvae. It was accordingly concluded that no advantage was to be gained either by adjusting the *p*H of the saline or by the addition to it of bicarbonate.

6. *The Effect of Carbohydrates on the Larvae.*—Different workers on salines have at different times advocated the addition of glucose to the solution. Willmur (1911) working on salines for the culture of fibroblasts, stressed its importance as an agent responsible for stimulating them to migration. Lapage (1935) records that the addition of glucose, galactose, lactose and laevulose to the medium had little effect on the fourth stage larvae of Trichostrongylids either under sterile conditions or in the presence of bacteria.

The function of glucose in a saline is problematic. Willmur's work seems to indicate that as far as fibroblasts are concerned, its function is that of an energy source. Lapage's experiments on the other hand, however, indicate that as far as nematode larvae are concerned, it is without function. Further support for this view is afforded by the findings of Stannard, McCoy & Latchford (1938) that the addition of glucose to the medium did not affect the metabolism of the larvae of *Trichinalla spiralis*.

A series of experiments was performed with the object of determining the effect (if any) of glucose on the larvae. Concentrations of 1% and under had no apparent effect, but increase in the concentration above this resulted in the manifestation of an apparently toxic effect. In no case was there any beneficial effect apparent. There does not therefore appear to be any reason for including it in the saline, and the original saline was therefore retained as a standard basis for culture media.

Conclusions.

(1) The most suitable antagonised saline for the larvae with regard to total ionic concentration and efficient antagonism, was found to contain 0·80% NaCl, 0·020% KCl, 0·020% $CaCl_2$ and 0·010% $MgCl_2$. The life of the larvae in this was approximately 5½ days. Small variations, either in the total concentration of the solution or in the proportions of the various constituents did not appear to have any serious effects on the larvae.

(2) The larvae did not appear to be affected by variations in the *p*H of the medium between *p*H 5·0 and *p*H 9·0. It was not therefore considered necessary to adjust the *p*H of the foregoing medium which usually

fell in the neighbourhood of 7·0–7·3. As would be expected from the foregoing, the addition of sodium bicarbonate to the solution did not affect its suitability as a medium and was therefore not included.

(3) The addition of glucose to the solution did not affect the larvae in any way as long as the concentration remained below 1% ; above this concentration it appeared to have a toxic effect.

(4) In view of these results it was finally decided that the composition of saline mentioned in paragraph 1 of this section was the most suitable in all respects as a basis for culture media for the larvae of *Ascaris suum*.

Acknowledgments.

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A New Species of *Strongyloides* from the Cat.

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INTRODUCTION.

THE first report of a species of *Strongyloides* parasitic in cats appears to be that by Chandler (1925 and 1925a). Sandground (1925b) found that cats could be infected with *Strongyloides stercoralis* from the dog but infections were light and were retained for short periods only. Later (1928) he found that cats had a slightly greater susceptibility to the human strain of *S. stercoralis* than dogs.

In view of the fact that many species of the genus *Strongyloides* are defined only by measurements and poorly defined morphological characters, careful consideration was necessary before the formation of a new species. The parasite described in this paper, however, seems to have such morphological features as to allow its status as a new species. Accordingly, the new species *Strongyloides cati* has been formed. Though the present form has been retained as a parasite in the domestic cat for 12 years, it was originally found by Professor R. T. Leiper in the " Rusty Tiger Cat " (*Felis planiceps*, Malay States).

The Free Living Generation.—In faecal culture at 27°C. the eggs passed by the parasitic females hatched and gave rise to rhabditiform larvae. These grew rapidly and in two or three days a large proportion of them developed into rhabditiform males and females, the remainder developing directly to infective larvae. After being fertilised the females passed eggs, or later, active rhabditiform larvae which in turn gave rise to the second generation of infective larvae. These were usually found in culture after 4 or 5 days. It has been found, under the culture conditions stated above, that a few of the second generation rhabditiform larvae developed into males similar to those of the first generation. No second generation females were noted.

The nature of the free living cycle may be summarised as mixed but predominantly indirect.

The Rhabditiform Larvae.—Measurements, total length, ·27–·52 mm. (average ·34 mm.) ; *α, 17·4–26·8 (average 20·1) ; β, 3·4–4·75 (average 4·1) ; γ, 4·0–8·3 (average 6·4).

No marked differences between the larvae arising from parasitic or free living females could be detected. The variation in measurements of both types of larvae was so great that size could not be regarded as a basis for diagnosis. Spears averaging 14μ in length could usually be seen in the oseophagus. They were most obvious in young freshly fixed material. In young larvae the mouth cavity was tubular, becoming globular in aged larvae.

Immediately after escaping from the free living female the larvae were about 280μ in length. The mouth cavity was tubular and well chitinised and the primordial sex cell very small. With age this latter became fairly obvious. The increase in the length of the oesophagus as the larvae developed into adult free living males or females was relatively greatest in the section second from the anterior end.

The Rhabditiform (Free Living) Females.—Measurements, total length, ·85–1·48 mm. (average 1·04 mm. ; α, 18·8–22·0 (average 20·8) ; β, 7·2–7·7 (average 7·4) ; γ, 12·0–10·8 (average 11·3).

Young females, found after 2 days in faeces cultured at 27°C., laid eggs, but older worms contained active larvae. The eggs were unevenly shaped and varied greatly in size, those containing mature larvae being much larger than those laid by young worms and usually very broad at one end. From 3 to 13 eggs were observed in uteri of different worms. More mature females contained from 3 to 25 larvae. The vulva was simple in character and, except in the case of very young worms, was covered with a dark sticky substance, probably secreted from the cement glands of the male. It was situated slightly anterior to the mid point of young worms, slightly posterior in old ones. There was no marked reduction in diameter immediately behind the vulva and the tail tapered

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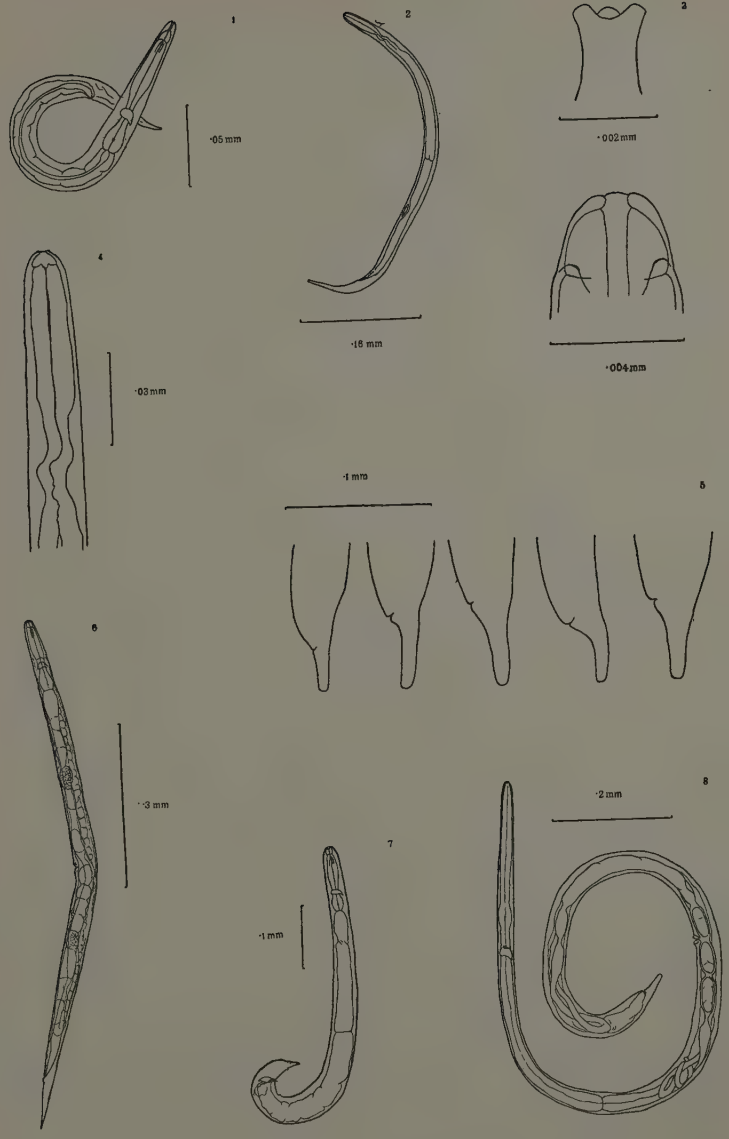
* $\alpha = \frac{\text{Total length}}{\text{Width (greatest)}}$; $\beta = \frac{\text{Total length}}{\text{Oesophagael length}}$; $\gamma = \frac{\text{Total length.}}{\text{Tail length.}}$

Fig. 1.—Rhabditiform larva immediately after escaping from the free living female.
Fig. 2.—Larva from the lung of the cat 4 days after cutaneous infection.
Fig. 3.—Head and tail ends of the filariform larva.
Fig. 4.—Anterior end of larva from lung of the cat 4 days after cutaneous infection.
Fig. 5.—Showing the variation in the shape of the tail in the parasitic female.
Fig. 6.—Young free living female.
Fig. 7.—Young free living male.
Fig. 8.—Adult parasitic female.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evenly to a point. The cuticle showed a distinct annulation. Attempts to locate the ventral gland were unsuccessful. The shape of the mouth cavity varied according to age, being narrower and more complex in old worms.

The most notable feature of the rhabditiform females was the structure of the oeseophagus (see Figure 9) which was divided into several sections. The short tail (see Table) and the three spears averaging 27·8μ in length, situated in the second section (from the anterior end) of the oesophagus were other noteworthy characters.

The Rhabditiform (*Free Living*) *Male.*—Measurements, total length, ·57–·9 mm. (average ·74 mm.); α, 17·8–24·0 (average 19·5); β, 5·3–6·6 (average 6·1); γ, 9·5–12·0 (average 11·0).

Young males were found in culture shortly before the appearance of females. They were stout worms with a hook shaped tail end. A ventral pre-anal papilla and two pairs of post-anal papillae were found (see Figure 14). The former was always prominent and was situated at an average distance of 31·6μ from the anus. One pair of post-anal papillae were on the ventral surface averaging 35·2μ from the anus; the other pair were placed dorsally about 13·6μ nearer the tip of the tail. At times the former pair were very large but the latter were always small. The spicules, which averaged 41·55μ in length, were well chitinised with large convoluted heads and when dissected out showed narrow alae (see Figure 12). The gubernaculum was of the characteristic *Strongyloides* type but had a heavily-chitinised finger-like process extending towards the tip of the spicules. The length of the gubernaculum averaged about 24·5μ. In examining the females the spicules and gubernaculum were occasionally found embedded in the cement surrounding the vulva.

Two sac like structures in close proximity to the heads of the spicules could be clearly seen in young males. These were possibly the glands which secreted the cement found around the vulva of the female. "

The oesophagus was similar to that in the female but was somewhat shorter in proportion to the body length. Oesophageal spears were present, averaging 22·1μ in length. In no case could the ventral gland be discerned. The cuticle was clearly annulated.

The Filariform (*Infective*) *Larvae.*—Measurements, total length, ·49–·67 mm. (average ·57 mm.); α, 35·0–46·2 (average 39·2); β, 2·1–2·5 (average 2·2); γ, 8·7–8·0 (average 8·3).

Table showing Measurements of Closely Allied Species.

	I. Cat (Chandler).	Cat (Rogers).	II. Dog.	III. Monkey.
Rh. ♀				
L.	1–1·2 mm.	0·85–1·48 mm. (1·04)	0·75 mm.*	0·75–0·85 mm. (0·79)*
α	25·6–26·1	18·8–22·0 (20·8)	21·4	13·6–17·5 (16·2)
β	7	7·2–7·7 (7·4)	6·2	5·9–8·9 (6·6)
γ	10·6–10·9	10·8–12·0 (11·2)	6·8	6·5–8·2 (7·3)
Sp.	?	23–28μ (27·8)	16–19μ	31–38μ
Rh.♂				
L.	0·57–0·98mm.	0·57–0·9 mm. (0·74)	—	0·40–0·74 mm. (0·63)*
α	22·5	17·8–24·0 (19·5)	—	13·9–23·5 (19·9)
β	6·5	5·3–6·6 (6·1)	—	3·6–6·1 (5·2)
γ	12·4–14·2	9·5–12·0 (11·0)	—	7·3–11·7 (9·9)
Sp.	?	21·7–23·2 (22·1)	—	?
Spi.	32–36μ	32·7–44·0μ (41·5)	—	25–35μ (30·5)
Gub.	21–22·5μ	23·4–25·6μ (24·5)	—	15μ approx.
Pap.	Pr. Pre-anal only	Med. Pre-anal. Two Prs. Post-anal.		Med. Pre-anal. One Pr. Post-anal.
Fil. Larvae.				
L.	0·52–0·61 mm. (0·58)	0·49–0·67 mm. (0·57)	0·576 mm.*	0·44–0·67 mm. (0·55)*
α	37·5–38·4	35·0–46·2 (39·2)	16·6	16·4–24·3 (19·9)
β	2·3	2·1–2·5 (2·2)	4·2	2·9–5·0 (3·8)
γ	?	8·7–8·0 (8·3)	6·0	5·7–8·0 (6·6)
Paras. ♀ ...				
L.	2·6–2·92 mm.	2·37–3·33 mm. (2·8)	1·8 mm.*	3·17–4·01 mm. (3·69)*
α	65·0–66·7	61·8–79·5 (69·6)	48·5	63·4–72·8 (67·8)
β	About 4	3·75–4·15 (3·98)	3·2	4·2–5·4 (4·9)
γ	28·1–41·2	61·30–87·5 (73·8)	35·9	48·7–68·3 (59·8)
V.	66·6–68·0%	65·2%	67·8%	63·8%
T.	*S. stercoralis*	*S. papillosus*	*S. stercoralis*	*S. stercoralis*

L.=Total Length ; Sp.=Length of Spears ; Spi.=Length of Spicules ; Gub.=Length of Gubernaculum ; Pap.=Types of Papillae ; V.=The Position of Vulva as the Percentage of the Total Length from the Anterior End ; T.=Type of Tail.

I, from Chandler (1925). II and III from Kreis (1932).

* Kreis gives these figures as 0·75μ, 0·750–0·858 (0·794)μ, etc. Obviously μ should be replaced by mm.

Figures given between brackets represent averages.

The infective larvae developed directly from the first rhabditiform stage or indirectly from the rhabditiform larvae arising from the free living females. No distinction could be drawn between these two types of larvae.

In general appearance the filariform larvae were of the typical *Strongyloides* type. The tails ended in a triradiate projection (see Figure 3). It did not seem to be a simple notch as figured by Kreis (1932) in the case of *Strongyloides simiae*. In agreement with Sandground (1925) it was found that this projection varied greatly in size and could not be considered to be of any diagnostic value. The mouth cavity was simple and tubular. At the level of the base of the mouth cavity, the head of the larvae bore several tooth-like structures. No spears could be detected in the oesophagus. The intestine contained numerous granules, many of which appeared to be fatty in nature, staining with Sharlach R. Though the excretory pore was clearly marked the ventral gland could not be detected.

The most notable characters of the infective larvae were the narrowness of the body and the length of the oeseophagus in proportion to the total length (see Table).

The Parasitic Generation.—Infection was successful by mouth or by skin. After 4 days larvae were found in the host's lungs. Young females were found in the small intestine 6 days after cutaneous infection. Eggs were recovered from the faeces 10 or 11 days after skin infection (Erhardt & Denecke, 1939, on one occasion have found eggs in the faeces 7 days after cutaneous infection) the whole cycle from egg to egg taking a minimum of about 14 days.

The Parasitic Stages in the Host's Lungs.—Measurements, total length, ·52–·55 mm. (average ·53 mm.); α, 27·8–32·5 (average 30·7); β, 2·0–2·3 (average 2·2); γ, 9·0–9·5 (average 9·15).

Four days after cutaneous infection larvae were collected from the host's lungs. They were somewhat similar to the infective stages, possessing the typical spiked tail. The most marked characteristic was the " S " in the oesophagus (see Figure 4). The young female worm, found in the intestine of the cat 6 days after cutaneous infection, had a similar " S " in the oesophagus.

Up to the present time parasitic males have not been found. It appears, however, that Kreis (1932) found them to be very rare in the

case of *Strongyloides* of the dog and man, and perhaps further investigation may reveal them in the cat.

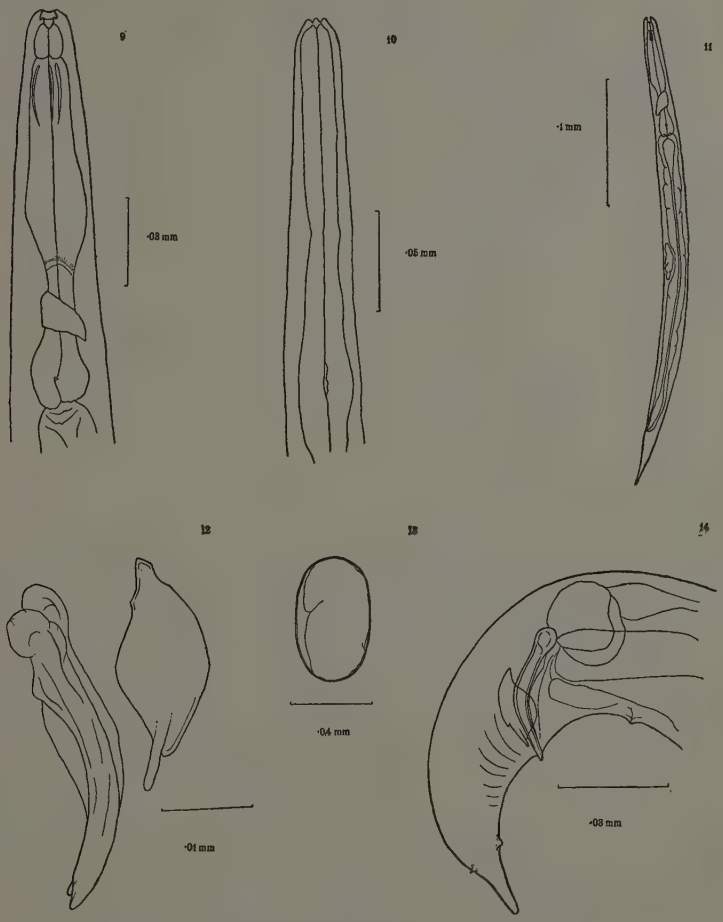

Fig. 9.—Anterior end of the free living female.
Fig. 10.—Anterior end of the parasitic female.
Fig. 11.—Old rhabditiform larva of the first generation.
Fig. 12.—Spicules and gubernaculum of the free living male.
Fig. 13.—Egg found in fresh faeces.
Fig. 14.—The tail of the free living male.

The Parasitic Female.—Measurements, total length, 2·37–3·33 mm. (average 2·80); α, 61·8–79·5 (average 69·6); β, 3·75–4·15 (average 3·98); γ, 61·3–87·5 (average 73·8).

Young females were found in the intestine of the cat 6 days after cutaneous infection. They were situated in the anterior portion of the small intestine up to within one foot from the pyloric valve. At the end of the first third of its length the oesophagus narrowed and then swelled into a small bulb (see Figure 8). No spears were present in the oesophagus. The head bore 3 distinct papillae. The vulva was about 65·2% of the body length from the anterior end. The ovaries were twisted. Immediately before the anus the tail narrowed markedly, ending in a blunt finger-like projection. The cuticle was indistinctly annulated. No ventral gland could be detected.

Eggs from the parasitic female in fresh faeces were always poorly developed and several hours' incubation at 27°C. was necessary before hatching occurred. The eggs measured 32·0–40·0μ (average 35·0μ) by 57·6–64·0μ (average 60·8μ).

Discussion.

Strongyloides cati differs from *S. stercoralis var. felis* Chandler, 1925, in several noteworthy features besides measurements.

1. Eggs, not larvae, are found in fresh faeces.

2. The parasitic female possesses the *S. papillosus* type of tail. Sandground (1925b) has criticised this character as a basis of diagnosis and gives camera lucida drawings to show the variation in the nature of the tail in various species of *Strongyloides*. Figure 5 shows the variation in the shape of the tail in *S. cati*. Comparison with Sandground's figures shows that the shortness and sudden tapering of the tail are fairly distinctive in the latter form.

3. Spears are present in the oesophagus of free living rhabditiform stages.

4. The male has two pairs of post-anal papillae.

5. The gubernaculum has a heavily chitinised distal finger-like process.

6. The parasitic females are not found within one foot of the pyloric valve. (*S. stercoralis var. felis* was found "immediately behind the pyloric valve.")

It appears from a figure drawn by Looss (1911 Pl. 14, Fig. 154) that the free living male of *S. stercoralis* has similar distribution of papillae to those on *S. cati*. The characters 1, 2, 3 and 5, however, seem to separate these two species fairly clearly.

S. fülleborni von Linstow, 1905, from the description added by Goodey (1926) can be clearly differentiated by the marked constriction in the body behind the vulva in the free living female and by the lack of oesophageal spears.

The characters 2, 4 and 5 serve to distinguish *S. cati* from *S. simiae* Lü & Hoeppli, 1923, which Kreis (1932) has described in some detail.

It appears that *S. cati* has features of both the *S. stercoralis* and *S. papillosus* groups (Chandler 1925). Of the former group it has the features :–parasite of carnivorous animals, mouth with 3 fairly prominent lips, tail of female of the free living generation less than $\frac{1}{10}$ of the body length. The characters of the latter group are :–body sharply constricted behind the anus and ending in a short finger-like tail, bluntly rounded at the tip, eggs never hatch before leaving the host, and the tail of the male bears post-anal papillae.

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